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COMMERCIAL ORGANIC ANALYSIS

COMMERCIAL ORGANIC ANALYSIS;

A TREATISE ON THE

ROPERTIES, PROXIMATE ANALYTICAL EXAMINATION, AND MODES OF
ASSAYING THE VARIOUS ORGANIC CHEMICALS AND PRODUCTS
EMPLOYED IN THE ARTS, MANUFACTURES, MEDICINE, &c.;

WITH CONCISE METHODS FOR

THE DETECTION AND DETERMINATION OF THEIR IMPURITIES,
ADULTERATIONS, AND PRODUCTS OF DECOMPOSITION

BY

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Second Edition, Revised and Enlarged

VOLUME III.—PART III.

*GETABLE ALKALOIDS (Concluded), NON-BASIC VEGETABLE BITTER
PRINCIPLES, ANIMAL BASES, ANIMAL ACIDS, CYANOGEN
AND ITS DERIVATIVES*

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PREFACE TO VOLUME III.—PART III.

THE instalment of "COMMERCIAL ORGANIC ANALYSIS" now published is nominally PART III. of VOLUME III., though practically it forms VOLUME V. of the book. It was intended to conclude the work with this issue, but to have done so would have rendered the volume unwieldy and have further delayed its publication. Hence it has been thought better to publish the matter already in type at once, reserving the consideration of PROTEIDS AND ALBUMINOID COMPOUNDS for a concluding Volume, which I hope will be issued before the end of the current year.

The Part now published treats of the less important Vegetable Alkaloids, left over from PART II.; Non-basic Vegetable Bitter Principles; Animal Bases, including Ptomaïnes; Animal Acids; and Cyanogen Compounds. With the exception of the last Chapter, revised and enlarged from that published in 1879, the whole of the subject-matter is new. The information has been compiled with care from a great variety of sources, and a large number of the tests and methods have been carefully investigated; but the rarity and difficulty of preparing many of the compounds described has rendered it impossible to verify certain of the processes.

I am fully conscious that much of the matter is scarcely such as might be expected to be contained in a work purporting to treat of Commercial Analysis, but I have thought it better to include all facts possessing for me an analytical or practical interest, believing that what I find useful myself will also be of value or interest to others.

I am indebted to Mr R. A. Cripps, Dr James Edmunds, Mr Ernest J. Parry, Mr A. Gordon Salamon, Mr F. W. Keating Stock, Mr Francis Sutton, Mr R. Wright, and other friends for perusal and correction of certain of the articles, and express my sincere thanks for the services they have rendered me in this connection.

I desire also to acknowledge the zealous assistance rendered by Mr Arnold R. Tankard in the investigation of a large number of the tests and processes described, in the correction of the proof-sheets, and in the compilation of the Index.

ALFRED H. ALLEN.

67 Surrey Street,
Sheffield, *30th June* 1896.

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VEGETABLE ALKALOIDS.

(*Continued.*)

The more important of the vegetable alkaloids have already been considered (Part II. pages 127 to 572). There remain a number of vegetable bases which are of interest or importance from their employment in medicine (*e.g.*, emetine, physostigmine, pilocarpine); their marked poisonous character (*e.g.*, gelsemine, colchicine); or their occurrence in condiments (*e.g.*, piperine, sinapine). These will be considered approximately in alphabetical order. Many other alkaloids exist of which little is known, or which are not of sufficient interest or practical importance to require description. In an appendix to the Chapter will be found a description of the more important non-basic bitter principles of vegetable origin.

Alkaloid of Papaya.

Carpaine, $C_{14}H_{25}NO_2$, is contained in the leaves of the Papaya or Papaw tree (*Carica Papaya*) of Java,[1] from which it was first isolated by M. Greshoff (*Ber.*, xxiii. 3537; *Pharm. Jour.*, [3], xxi. 560).[2]

---

[1] The same plant yields the vegetable ferment or trypsin called Papain, which has recently attracted much attention owing to its remarkable digestive action on proteids. (See *Pharm. Jour.*, [3], xxiv. 183, 207, 633, 705, 757, 758, 845, 831, 1005, 1088; xxv. 183.)

[2] Carpaine is best extracted by digesting the finely-powdered dried leaves of the plant with hydrochloric acid, and subsequently with alcohol. The extract is evaporated to a syrup, treated with acidulated water, filtered from the residue of chlorophyll, &c., and shaken with ether to remove resin. The aqueous layer is then made alkaline with soda, and the carpaine shaken out with ether. The yield ranges from 0·08 to 0·25 per cent. (in young leaves).

According to J. J. L. van Ryn (*Arch. der Pharm.*, ccxxxi. 184; *Jour. Chem. Soc.*, lxiv. i. 740), carpaine forms colourless, anhydrous lustrous prisms, melting at 120° to 121° (not at 115°, as stated by Greshoff), and sublimes partially at a somewhat higher temperature. It is practically insoluble in water and in solutions of caustic and carbonated alkalies. It dissolves readily in absolute alcohol (1 : 9) and in amylic alcohol, but is very sparingly soluble in dilute spirit. It is soluble in chloroform and carbon disulphide in all proportions, and readily in benzene, but only sparingly (1 : 100) in petroleum ether. It is but sparingly soluble in ether when once crystallised.

Carpaine is dextro-rotatory, $[\alpha]_D$ being $+ 21{\cdot}55°$ and unaffected by concentration.

Carpaine has an extremely bitter taste, perceptible in dilutions of 1 in 100,000. Carpaine has a strong depressing action on the heart, and has been employed in medicine, being, according to van Oefele, the only substitute for digitalis (with the exception of the members of the caffeine group) which does not cause irritation or suppuration when injected hypodermically (*Pharm. Jour.*, [3], xxxiii. 1). It acts as a heart poison both on frogs and birds.

Carpaine is not removed from acidulated solutions by ether or chloroform. Its solutions are alkaline to litmus and cochineal, but have no action on phenolphthalein.

Carpaine yields crystallisable salts. B,HCl forms shining needles, readily soluble in water. B_2,H_2PtCl_6 is yellow and crystalline. The alkaloid is precipitated from very dilute solutions by Mayer's reagent, phospho-molybdic acid, tannin, picric acid, and potassium thiocyanate. $B,HAuCl_4$ crystallises in lemon-yellow needles, melting at 205°. Bromine-water and iodised potassium iodide give precipitates even with an aqueous solution of carpaine.

With strong mineral acids, either alone or with oxidising agents, carpaine gives no colour-reactions; except that a mixture of sulphuric acid and potassium dichromate is turned green.

Carpaine appears to be a secondary base, since its compound with ethyl iodide is decomposed by potash forming ethylcarpaine, $C_{14}H_{24}(C_2H_5)NO_2$, which crystallises from dilute alcohol in colourless silky needles, melting at 91°. Carpaine is not acted on by either benzoyl chloride or acetyl chloride. With nitrous acid it forms a nitroso-derivative, $C_{14}H_{24}(NO)NO_2$, which crystallises in colourless prisms, melting at 144° and soluble in alcohol.

By the oxidation of carpaine with sulphuric acid and potassium permanganate, ammonia and an acid free from nitrogen are obtained.

Alkaloids of Colchicum.

The meadow saffron, *Colchicum autumnale*, contains a poisonous alkaloid called colchicine. The decomposition-product colchiceïne (page 6) probably occurs naturally in many cases.

Colchicine $(CH_3O)_3.C_{15}H_9(NH.C_2H_3O).CO_2.CH_3$, is present in all parts of the colchicum plant, but chiefly in the seeds.

The partial synthesis of colchicine has been effected by heating colchiceïne, methyl iodide, and sodium methylate together at 100°. Methyl-colchicine is formed at the same time.[1] Colchicine is a yellowish-white or bright yellow substance, which darkens on exposure to light, and melts with decomposition at 143° to 147°. It dissolves slowly but abundantly in water, forming an intensely bitter, lævo-rotatory solution. It is readily soluble in alcohol, and is also dissolved by chloroform,[1] benzene, and amylic alcohol. It is insoluble in absolute ether and in petroleum spirit.

The basic characters of colchicine are very feebly marked. It is neutral to litmus, and most of its salts are decomposed by water. Hence it is extracted by suitable immiscible solvents, both from acidulated and from alkaline solutions.

In presence of a dilute mineral acid or alkali, solutions of colchicine gradually become intensely yellow. Concentrated acids yield a yellow resinous precipitate. Concentrated nitric acid (sp. gr. 1·42) colours colchicine violet-blue, the tint changing to yellow, and ultimately to green. If the violet solution be diluted with water it turns yellow, and changes to a fine orange or red on adding excess of soda. With very minute quantities of colchicine the coloration with nitric acid is rose-red. The reaction is extremely delicate. Sulphuric acid dissolves colchicine with intense yellow colour, and on adding a drop of nitric acid a dark brown spot is formed, passing gradually through violet and brown to yellow. In an aqueous solution of colchicine, chlorine and bromine water occasion a yellow precipitate which dissolves in ammonia with orange colour.

Colchicine is precipitated very perfectly by gallo-tannic acid and by phospho-molybdic acid. The latter reagent is a useful one for separating colchicine from solutions containing it. The precipitate gives the foregoing colour-reactions with acids, but if preferred the free base may be obtained by agitating the precipitate with ammonia and chloroform, and evaporating the chloroformic solution to dryness.

---

[1] Colchicine is stated to form with chloroform a compound of the formula $C_{22}H_{25}NO_6$, $2CHCl_3$, which crystallises in needles, and is gradually decomposed by boiling with water. Methyl-colchicine forms no similar compound, upon which fact Teisel founded a separation of the two bases. R. Wright states that the chloroform compound of colchicine must be very readily decomposed by water, if it exists.

If an aqueous solution of colchicine be treated with powdered manganese dioxide and dilute sulphuric acid, and the liquid filtered after some hours, a filtrate is obtained which acquires a full blue colour on adding excess of ammonia.

An aqueous solution of colchicine gives a brown or yellow precipitate with iodised potassium iodide, and may be conveniently isolated by that reagent. On treating the precipitate with sodium thiosulphate (hyposulphite) the alkaloid dissolves, and may be extracted from the alkalised solution by chloroform.

Colchicine is precipitated by potassio-iodide of bismuth, but not by Mayer's reagent nor by mercuric chloride, unless a mineral acid be added, when the former reagent produces a copious lemon-yellow precipitate. Potassio-iodide of cadmium reacts similarly.

Colchicine solutions give no precipitate with platinic chloride, but yield with auric chloride a yellow amorphous precipitate containing $B,HAuCl_4$, which rapidly becomes crystalline, and is readily soluble in alcohol.

An alcoholic solution of colchicine gives a garnet-red coloration with ferric chloride. An aqueous solution gives no immediate reaction, but on warming a green coloration is produced, probably owing to the formation of colchiceïne. In presence of much hydrochloric acid the coloration produced by ferric chloride on heating varies from green to greenish-black. If the liquid be shaken with chloroform after cooling, the latter becomes coloured brownish, garnet-red, or dark and opaque.

An aqueous solution of phenol gives a strong milky turbidity with colchicine, and afterwards a yellow resin is precipitated. Acids prevent the reaction.

Picric acid produces no precipitate in a neutral solution of colchicine. On adding dilute sulphuric acid a resinous precipitate is formed, which attaches itself to the sides of the vessel.

E. Barillot (*Bull. Soc. Chim.*, 1894, p. 514; *Jour. Chem. Soc.*, 1895, abs. ii. 300) has described the following mode of operating, which he claims to afford a very reliable test for colchicine, and a means of distinguishing it from morphine and codeine. A minute quantity of the free alkaloid, in the form of an ether or chloroform residue, is mixed very intimately with 0·25 gramme of oxalic acid and 1 c.c. of strong sulphuric acid. The mixture is sealed up in a small glass tube, which is kept at 120° C. for one hour. Subsequently the tube is opened, the colour of the mixture observed, and excess of alcoholic soda added. Operating in this way, the following results are stated to be obtained:—

Alkaloid.	Coloration in the Cold.	Coloration after Heating.	Observations.
Colchicine,	Golden yellow.	Dark reddish-brown, not modified by addition of water.	On treating the aqueous solution with an alkali and reacidifying, a yellow precipitate is obtained, which is soluble in chloroform;[1] on evaporating this solution a yellow residue is obtained, which becomes violet-red on treatment with nitric acid (sp. gr. 1·42) and raspberry-red with strong sulphuric acid.
Morphine,	Light blue.	Reddish-brown.	On addition of a large volume of water the colour sometimes changes into blue. Treating this solution with caustic potash and alcohol, then acidifying it, and shaking it with chloroform, the latter assumes a blue colour. Ether takes a purplish-red colour. On evaporating these two solvents morphine-blue remains behind.
Codeine,..	Bright blue.	Greenish-blue.	Like morphine.

Barillot states that none of the ptomaïnes shows any similar reaction. With 1 mgrm. of colchicine the above reaction can be repeated ten times.

Colchicine is very unstable, being decomposed on heating with alkalies or dilute acids with elimination of methyl alcohol and formation of colchiceïne. This latter substance is formed so readily that some of the reactions commonly attributed to colchicine itself are probably due to its decomposition-product.[2] By further treatment, as by increasing the strength and proportion of the acid, the colchiceïne itself undergoes further change, with elimination of an acetyl and one or three methyl groups, producing compounds which, together with the higher terms of the series, are formulated on next page.

These decomposition-products have some importance in connection with the detection of colchicine, as in the course of its isolation in toxicological investigations its partial conversion into some of the simpler members of the series is liable to occur.

---

[1] If the colouring matter is not entirely taken up by the chloroform, but floats about in flakes, it may be collected on a very small filter, and the latter dried, cut into strips, and immersed in the acid.

[2] N. Rossenwasser (*Pharm. Jour.*, [3], viii. 507) states that colchicine is not precipitated from aqueous solutions or solutions acidulated with an organic acid by iodine, auric chloride, or by Mayer's, Scheibler's, or Sonnenschein's reagent; but that each of these gives a precipitate after the solution has been acidulated with oxalic acid or a mineral acid, or boiled for a few minutes with acetic acid.

Colchic acid. Colchicinic acid,	$(OH)_3 \vdots C_{15}H_9 \begin{cases} NH_2 \\ CO.OH \end{cases}$
Dimethylcolchic acid,	$(O.CH_3)_2(OH) \vdots C_{15}H_9 \begin{cases} NH_2 \\ CO.OH \end{cases}$
Trimethylcolchic acid,	$(O.CH_3)_3 \vdots C_{15}H_9 \begin{cases} NH_2 \\ CO.OH \end{cases}$
Acetyl-trimethylcolchic acid. Colchiceïne,	$(O.CH_3)_3 \vdots C_{15}H_9 \begin{cases} NH.C_2H_3O \\ CO.OH \end{cases}$
Methyl acetyl-trimethylcolchate. Colchicine,	$(O.CH_3)_3 \vdots C_{15}H_9 \begin{cases} NH.C_2H_3O \\ CO.O(CH_3) \end{cases}$
Methyl-colchicine,	$(O.CH_3)_3 \vdots C_{15}H_9 \begin{cases} N(CH_3).C_2H_3O \\ CO.O(CH_3) \end{cases}$

Colchiceïne, or Acetyl-trimethylcolchic acid, $C_{21}H_{23}NO_6$, is a body possessing both acid and feebly basic characters. It is best obtained by warming colchicine with water containing 2 per cent. of H_2SO_4 or 1 per cent. of HCl. On cooling, the colchiceïne separates in shining white needles, containing ½ aqua. It becomes anhydrous at 140°-150° and melts at 166°. It is readily soluble both in acids and alkalies, with yellow colour. Colchiceïne is soluble in hot water and very soluble in alcohol and chloroform; but insoluble in ether and benzene. The solutions of colchiceïne are lævorotatory. They are coloured yellow by alkalies and mineral acids, and concentrated nitric and sulphuric acids behave as with colchicine. The solution in hydrochloric acid reacts for the most part like colchicine. If sufficiently concentrated, auric chloride throws down an orange-coloured aurichloride, $B,HAuCl_4$, crystallising in needles. Bromine-water, phenol-water, and phosphomolybdic acid give slight precipitates with aqueous solutions of colchiceïne, but most other alkaloidal reagents give negative reactions. Precipitates are produced by acetates of lead and copper. Ferric chloride gives a fine green coloration with a dilute acidulated solution of colchiceïne.

When colchiceïne (or colchicine) is heated in the water-bath with 3 or 4 parts of hydrochloric acid of 1·15 sp. gravity, acetic acid is evolved, and a mixture of the hydrochlorides of colchic acid and its di- and tri-methylated derivatives is obtained. When a trial portion of the solution ceases to become turbid when mixed with water, the product is diluted with a little water and shaken twice with chloroform, which extracts the hydrochloride of trimethylcolchic acid and any unchanged colchiceïne only. The chloroform solution is evaporated, the residue taken up with water, and the separation of unchanged colchiceïne promoted by adding a crystal of that substance. The filtered solution is again shaken with chloroform, which now takes up colchiceïne only, and the aqueous liquid leaves the hydrochloride of trimethylcolchic acid on evaporation. On treating the last aqueous solution with an amount of caustic potash sufficient to react with the hydrochloric acid only, free trimethylcolchic acid separates on cooling.

TRIMETHYLCOLCHIC ACID, $C_{16}H_{12}(CH_3)_3NO_5+2H_2O$, forms minute yellow prisms, melting at 159° C. It closely simulates colchiceïne in its reactions. Ferric chloride produces a garnet-red coloration with green dichroïsm. On further addition of the reagent or of hydrochloric acid, the colour becomes green, and on shaking with chloroform the same reaction is obtained as with colchicine. Trimethylcolchic acid forms a *hydrochloride*, crystallising in glistening, yellowish-white plates, moderately soluble in cold water to form a yellow solution. B_2,H_2PtCl_6+2aq forms small yellow needles, and $B,HAuCl_4$ a brown precipitate. The solutions also give precipitates with chlorine-, bromine- and iodine-water, picric acid, and cadmium iodide.

DIMETHYLCOLCHIC ACID, $C_{16}H_{13}(CH_3)_2NO_5+4\frac{1}{2}H_2O$, produced together with the last substance, forms minute yellow prisms, melting at 141°. $B,HCl+aq$ forms sparingly soluble, white, microscopic needles. Its solution is precipitated by most of the alkaloidal reagents, except platinic chloride and tannin. It dissolves in concentrated sulphuric acid with orange colour, and on addition of a minute quantity of a nitrate gives the same colour-reactions as colchicine. With ferric chloride it behaves like trimethylcolchic acid.

COLCHIC ACID, or COLCHICINIC ACID, $C_{16}H_{15}NO_5$, is the final product of the action of strong acid on colchicine, and is obtained by heating that body or colchiceïne with four parts of fuming hydrochloric acid to 140° C. The resultant hydrochloride is very soluble in water, and yields the free "acid" on fractional neutralisation with caustic potash. Colchic acid forms brown flakes, the solution of which is precipitated by most of the alkaloidal reagents. With ferric chloride the solution in hydrochloric acid gives an intense brownish-red colour, which is not taken up by chloroform. On adding a trace of potassium nitrate to the solution in concentrated sulphuric acid, a dull red colour is produced, which changes to a splendid red colour on the addition of excess of ammonia.

ASSAY OF COLCHICUM. DETERMINATION OF COLCHICINE.

The parts of the meadow saffron which contain colchicine in largest proportion are the seeds and the root or corm. The British Pharmacopœia directs the employment of the seeds for the preparation of the *tincture* (which is made with proof-spirit), and the corm for that of the *extract* or *wine* of colchicum.

In addition to colchicine, which is the only important medicinal constituent, colchicum *corms* or *tubers* contain starch (about 10 per cent.), gum, sugar, tannin, and fatty, resinous and colouring matters. The *seeds* contain much sugar, a small proportion of an acid resembling gallic acid, and from 6 to 8 per cent. of fixed oil. Colchiceïne is probably present in many cases.

J. Hertel (*Year-Book Pharm.*, 1882, page 78) recommends, for the extraction of colchicine, that the uncrushed seeds should be exhausted with 85 per cent. alcohol.[1] Powdered seeds are stated to give an extract loaded with foreign matter and to yield less alkaloid. The solution is shaken with calcined magnesia, filtered after a few hours, and the filtrate distilled in a vacuum. The extract is diluted with about 10 measures of water, filtered from oily matter, and repeatedly shaken with chloroform. The chloroform is separated, distilled off, and the syrupy residue poured on glass plates and heated to 80°–100° C. for an hour, or until it is no longer soft and waxy when hot (indicative of the presence of chloroform, which is retained very obstinately). The brown brittle mass thus obtained is dissolved in 20 parts of water, and the filtered solution evaporated to dryness. The yield of alkaloid obtained by Hertel from colchicum seeds, working in this manner, was from 0·38 to 0·41 per cent.

For the preparation of colchicine, Huber exhausts the seeds with boiling alcohol, and dilutes the resultant solution with 20 measures of water to precipitate fatty matters. The filtered liquid is treated with basic acetate of lead, and the excess of lead removed by sodium phosphate. The colchicine is precipitated from the filtrate by tannic acid, which is said to form a compound of definite composition. The moist precipitate is mixed with oxide of lead and the mass treated with boiling alcohol, which on evaporation leaves the colchicine as a yellow bitter powder.

For the *assay* of colchicum seeds, Farr and Wright recommend that 20 grammes of the powdered material should be exhausted by cold percolation with spirit of 50 per cent., 25 c.c. of water added to the extract, and the alcohol evaporated at a moderate temperature. The residual liquid is diluted to 20 c.c., and when quite cold slightly acidulated with sulphuric acid (5 c.c. $\frac{N}{10}$ H_2SO_4). It is then twice agitated with petroleum-ether to remove oil, and colouring matter, and the separated aqueous liquid made slightly ammoniacal and agitated twice with chloroform, the separated chloroform evaporated, and the residual alkaloid dried at 100° and weighed.[1] The proportion of alkaloid found in the seeds by Farr and Wright (*Pharm. Jour.*, [3], xxi. 957) by this process ranged from 0·46 to 0·95 per cent. This variation in alkaloidal strength extends to the official tincture of colchicum, which causes an uncertainty as to its potency highly objectionable in the case of so active a preparation.

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[1] Other operators prefer proof-spirit to stronger alcohol, and insist on the necessity of using a hot solvent. R. A. Cripps (*Pharm. Jour.*, [3], xxii. 364) has shown that the amount of alkaloids extracted when uncrushed seeds are used is little more than one-fourth of the quantity obtained when the powdered drug is used.

Toxicology of Colchicum.

Colchicine has valuable medicinal properties, and in excessive doses is a powerful poison. The free alkaloid is not official, but is employed in the forms of extract or wine prepared from the corms, or of a tincture of colchicum seeds. Colchicum is the active ingredient of certain proprietary remedies for the treatment of gout, but is regarded with grave mistrust by many competent authorities, owing to the variable effects produced by it. These are probably in great measure due to the uncertain alkaloidal strength of the official tincture and other preparations. Colchicine augments the excretion of uric acid, and reduces the quantity contained in the blood; but its accumulation in the system and marked toxicity render great caution necessary in its administration. The elimination of colchicine from the system occurs chiefly by the kidneys, but is very slow; and hence small doses, not poisonous in themselves (0·00016 gramme per kilogramme of body-weight), may cause death within five days. Colchicine causes congestion in the articular extremities and the marrow of the bones.

According to Mairet and Combemale (*Compt. rend.*, civ. 439, 515), colchicine acts, according to the dose, either as a diuretic or as a purgative, and in excessive quantities is an irritant poison, affecting more especially the digestive canal and the kidneys, though capable of irritating any of the organs. Its therapeutic effects are the same whether administered subcutaneously or by the stomach, but in the former case the action is more rapid and the dose required is smaller. In proportion to their weight, men are three times more susceptible to colchicine than are dogs or cats, the total dose required to produce diuresis in man being 0·002 to 0·003 gramme, and for a purgative effect 0·005 gramme. The fatal hypodermic dose of colchicine for dogs and cats is 0·000571 gramme, and when taken internally 0·00125 gramme, per kilogramme of the weight of the animal.

Poisonous doses of colchicum occasion all the symptoms of violent gastro-intestinal irritation, including griping, vomiting, diarrhœa, prostration, and painful spasms of the limbs and trunk (but without tenderness of the abdomen), followed by resolution and collapse, without delirium or coma. On *post-mortem* examination, the most notable symptoms are lividity of the skin in depending parts, engorgement of the veins, dark pitchy blood in the lungs, brain and trunk, sometimes a dark-coloured injection of the gastro-intestinal mucous membrane, and more or less shedding of the intestinal epithelium. The kidneys are pale.

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[1] It might be well to treat the residue with a little water, and again evaporate and dry at 100° to ensure the decomposition of the compound of colchicine with chloroform. R. Wright considers this precaution unnecessary.

In searching for colchicum, it must be remembered that the active principle colchicine readily suffers decomposition by heating with alkalies or dilute mineral acids, and that it is extracted from both alkaline and acidulated aqueous liquids by agitation with chloroform, but not by petroleum-spirit, which latter solvent may consequently be employed to remove fatty and resinous matters. When isolated in a fairly pure state, the chemical reactions of colchicine and its decomposition-product colchiceïne are sufficient for the recognition of the poison.

For the identification of the alkaloid when thus isolated, Obolonski (*Zeit. Anal. Chem.*, xxix. 493) prefers the violet coloration with nitric acid; the reaction with Erdmann's reagent (Part ii. page 146), which gives, in succession, green, dark-blue, violet, and yellow colorations, turning to raspberry-red on adding alkali; and the green colour produced by sulphovanadic acid (Part ii. page 148). Obolonski states that colchicine is with difficulty destroyed by putrefaction of animal matter, and that the kidneys, bladder, and urine are best suited for toxicological examination.

A non-poisonous substance giving many of the reactions of colchicine has been found among the normal constituents of beer, being apparently derived from the hops (*Archiv. der Pharm.*, [3], viii. 411; *Pharm. Jour.*, [3], vii. 351. See further, *Zeitsch. anal. Chem.*, xvi. 116, 328).

Alkaloids of Laburnum and Furze.

Cytisine, $C_{11}H_{14}N_2O$,[1] is contained in the unripe seeds, bark, and other parts of the laburnum (*Cytisus laburnum*), and several other species of the same genus. It is identical with the alkaloid of furze (*Ulex Europæus*), sometimes called ulexine.

To isolate cytisine, A. Partheil (*Archiv. der Pharm.*, ccxxxi. 448) recommends that laburnum-seeds should be extracted with

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[1] $C_{20}H_{27}N_3O$, the formula formerly attributed to cytisine, has been disproved by Partheil and other recent observers, and the simpler expression confirmed by Raoult's method.

60 per cent. alcohol acidulated with acetic acid, the alcohol distilled off, and the extract dissolved in water. The solution is passed through a wet filter to separate oil and resin, and then precipitated by lead acetate. The filtered liquid is then rendered strongly alkaline by caustic potash, and shaken with chloroform or amylic alcohol. The separated solvent is agitated with dilute hydrochloric acid, and the acid solution evaporated to dryness. On treating the residue with cold absolute alcohol almost all the colouring matter is separated, and by repeatedly crystallising the residual cytisine hydrochloride from water it is obtained in colourless, well-defined crystals. On decomposing the salt with strong caustic potash and extracting with chloroform, the free alkaloid is obtained as a pale yellow oil, which quickly solidifies to a crystalline mass, and may be further purified by recrystallisation from absolute alcohol or boiling petroleum-spirit.[1]

The constitution of cytisine has not been definitely ascertained, but one of the atoms of nitrogen is in secondary combination, as shown by the behaviour of the base with methyl iodide, acetic anhydride, and nitrous acid. The other nitrogen atom is either in tertiary or quaternary combination; while the oxygen exists neither as hydroxyl nor methoxyl, no acetyl-derivative being obtainable from methyl-cytisine on treatment with acetic anhydride. On distillation of cytisine with soda-lime, a base of the formula $C_9H_{13}N$ is obtained, which shows the alkaloid to be a pyridine-derivative.

Cytisine forms large, transparent, odourless, anhydrous prismatic needles or laminæ, melting at 150°–153°, and said to be sublimable unchanged. It is extremely soluble in water, alcohol, and chloroform, but less readily in benzene, amylic alcohol, or acetone; and is nearly insoluble in ether, petroleum-spirit, or carbon disulphide. Benzene dissolves 1·26, and amylic alcohol 0·30 per cent. In aqueous solution, cytisine is strongly alkaline, and exhibits the optical activity $[\alpha]_D = -119{\cdot}6°$.

Cytisine is a strong base, displacing ammonia from its salts even in the cold. All the ordinary salts are crystallisable and soluble. B,HCl and $B(HCl)_2 + 2\frac{1}{2}aq$ have been described. Two chloroplatinates are known; B_2,H_2PtCl_6 forming pale yellow, lustrous, sparingly soluble plates or needles, and $B,H_2PtCl_6 + 2\frac{1}{2}aq$ golden yellow, tolerably soluble needles, which decompose when heated without previously melting. $B,HAuCl_4$ crystallises in short, reddish-brown needles, which melt with decomposition at 212°–213°.

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[1] From the ripe seeds of *Cytisus laburnum*, Partheil isolated about 1·5 per cent. of cytisine, while the leaves and fruit gave smaller amounts. Choline was also found, but the base soluble in ether mentioned by Gerrard and Symons could not be obtained.

Cytisine gives precipitates with the usual alkaloidal reagents. Bromine-water gives an orange-red precipitate in very dilute solutions (1 : 15,000), as also do phospho-molybdic and phospho-tungstic acids (1 : 30,000). The most characteristic reaction of cytisine and its salts is that with ferric chloride, which produces a red coloration. This is destroyed by hydrogen peroxide, but on then warming the liquid a blue coloration is immediately produced. This reaction is very characteristic, and is said to be produced by 0·00005 gramme of cytisine.

Another characteristic reaction of cytisine is that observed by Magalhaës, which consists in adding thymol to a solution of cytisine in concentrated sulphuric acid, and heating, when a yellow coloration, finally passing into intense red, is produced.

With strong sulphuric acid, cytisine affords no colour-reaction, but on adding potassium bichromate a yellow colour, changing to brown, is produced, or with nitric acid an orange-yellow. It does not reduce phospho-molybdic acid.

Cytisine possesses marked poisonous properties. A case is recorded of a child having been poisoned by milk from a cow which had fed on laburnum, though cytisine has been ascertained to be relatively innocuous to cattle. All parts of the laburnum contain cytisine, and cases are on record of poisoning by the seeds, flowers, bark, and twigs. Cytisine has been described as intermediate in its action between strychnine and curarine, but vomiting and diarrhœa are common. The pupil is often dilated.

In toxicological inquiries, cytisine is best isolated by extraction with chloroform from a solution containing excess of caustic alkali. The urine and vomit are the most likely to contain the alkaloid. Salts of cytisine are excreted by the kidneys within twenty-four hours, but if laburnum seeds or leaves have been employed, the elimination may take a longer time.

An alkaloid isolated by A. W. Gerrard from the Furze (*Ulex Europæus*) (*Pharm. Jour.*, [3], xvii. 101, 229; xix. 1029), and called by him ulexine, is regarded by Kobert, Partheil, and others (*Pharm. Jour.*, [3], xxi. 759) as identical with cytisine. Gerrard at first disputed the identity of the two bases (*Pharm. Jour.*, [3], xx. 1017), but is now satisfied of the fact (private communication).

Cytisine is present in the bark and young tops of furze, but in smaller proportion than in the seeds, from which Gerrard isolated

0·19 per cent., while Partheil (*Ber.*, xxiv. 634) obtained as much as 1 per cent.[1] The cytisine from furze causes clonic spasms in frogs, and when the hydrochloride is placed on the tongue it produces numbness, similar to but less powerful than that produced by cocaine.

Gerrard has obtained indications of the presence in furze-seeds of a second alkaloid which is soluble in ether.

SOPHORINE, an alkaloid contained in *Sophora tormentosa*, is regarded by Greshoff as not improbably identical with cytisine (*Pharm. Jour.*, [3], xxi. 559, 1056; xxii. 609).

Alkaloids of Stavesacre.

The seeds of *Delphinium Staphisagria* contain several optically inactive alkaloids, which have been recently re-investigated by Charalampi (*J. Pharm.*, [5], xxiii. 202; abst. *Jour. Chem. Soc.*, lx. 842).[2]

DELPHININE, $C_{31}H_{49}NO_7$, or $C_{22}H_{35}NO_6$, forms rhombic crystals melting at 192°, having a pungent burning taste, is irritant and purgative. Its alkaline reaction gives no colour-reactions with acids, but when mixed with 1 to 2 volumes of malic acid, and treated with sulphuric acid, it yields an orange-coloured mass, which after several hours becomes dark rose-red, and ultimately a dirty blue.

DELPHISINE, $C_{31}H_{50}NO_7$ (?), forms needles melting at 189·2°, less soluble than delphinine in ether. With sulphuric acid and bromine-water it gives violet coloration, and with sulphuric acid and sugar a yellowish-brown colour, changing to green on adding a little water.

DELPHINOÏDINE, $C_{25}H_{42}NO_4$, or $C_{42}H_{68}N_2O_7$, is an amorphous base, which remains in the ethereal solution after the separation of the crystallisable bases. It gives the same colour-reactions as delphisine.

STAPHYSAGRINE, contained in the residue after the separation of the preceding bases, is regarded by Charalampi as a mixture of four amorphous alkaloids. The product described under the same name by Marquis (1877) had the composition $C_{22}H_{33}NO_5$, melted at 90° C., was soluble in 200 parts of water and 850 of ether, and very freely in alcohol and chloroform. It gave with strong sulphuric acid a faint cherry-red colour; with fuming nitric acid, a blood-red; and with sulphuric acid and bromine-water, a transient reddish colour.

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[1] Coarsely powdered furze-seeds were extracted with alcohol acidified with acetic acid, the solution distilled, and the residue treated with hot water and filtered to separate oil and resin. The solution was precipitated with lead acetate, and the filtrate treated with excess of soda and extracted with chloroform.

[2] The characters ascribed to the *Delphinium* alkaloids by Charalampi do not agree with those of previous observers, and his formulæ for delphisine and delphinoïdine are very improbable, as they are not in accordance with the law that the sum of the H and N atoms should be an even number.

The alkaloids of stavesacre, especially delphinine and delphisine, are powerful poisons, resembling aconitine in their action. Delphinine is said to have been employed with success in neuralgia, ear-ache, and tooth-ache. The dose is stated to be ½ grain. The seeds of stavesacre or larkspur are no longer official, but are still much used, as also is an infusion made from the flowers.

Alkaloids of the Calabar Bean.

Eserine or Physostigmine, $C_{13}H_{17}N_2(OH)(CO)(N.CH_3)$; or $C_{15}H_{21}N_3O_2$. This base is the characteristic poisonous alkaloid of the Calabar bean (*Physostigma venenosum* or *P. faba*) of West Africa;[1] also known as the esere-nut, chap-nut, and ordeal bean.[2]

Eserine forms crystals, which when pure are colourless, but which often have a pale red tint. Eserine is commonly stated to soften at about 40°, melt at 45°, and redden and decompose below 100°; but according to Petit and Polonovsky (*Bull. Soc. Chim.*, 1893, ix. 1008), it melts at 105° to 106°.

Eserine is very slightly soluble in water, readily soluble in alcohol, ether, chloroform, benzene, and carbon disulphide; but insoluble in petroleum-ether.

Eserine is lævo-rotatory, the value for $[\alpha]_D$ being :—in chloroform solution, $-82°$; in 98 per cent. alcohol, $-89°$; and in benzene or toluene, $-120°$.

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[1] *Physostigma cylindrospermum* is probably occasionally substituted for the official bean.

[2] "The Pharmacognosy and Chemistry of Calabar Beans" forms the subject of an able paper by P. MacEwen (*Chem. and Druggist*, xxx. 193; *Pharm. Jour.*, [3], xvii. 641), who recommends the following process for the extraction of the alkaloids:—Digest the powdered bean with its own weight of a mixture of three parts of rectified spirit with one part of water for a day, then pack in a percolator, and percolate with the same alcoholic menstruum until the percolate on dilution with water gives only a slight opacity with Mayer's solution. The spirit is then evaporated, and the aqueous residue treated with basic lead acetate, to remove extractive and colouring matters. The liquid is filtered, the excess of lead removed by sulphuretted hydrogen, the alkaloid liberated by ammonium carbonate, and extracted by agitation with chloroform, which on evaporation leaves the alkaloids as a residue of pale amber colour, wholly soluble in dilute acid.

The aqueous solution of eserine is strongly alkaline to litmus, and precipitates ferric hydroxide from a solution of ferric chloride.

On exposure to air and light, an aqueous solution of eserine becomes red, and ultimately dark brown, with formation of a red, crystallisable colouring matter called rubeserine, soluble in chloroform. The formation of rubeserine has been attributed to absorption of atmospheric ammonia, and is greatly facilitated by the presence of alkalies, even the traces dissolved from glass being said to be effective. On treating the reddened solution with hypophosphorous acid, sulphurous acid, sulphuretted hydrogen, sodium thiosulphate, or nascent hydrogen, in presence of a trace of free acid, the liquid is decolorised.

With caustic alkalies and fixed alkaline carbonates, concentrated solutions of eserine salts give oily precipitates of the free base, but no precipitates are formed in more dilute solutions. On shaking the alkaline liquid with air, it rapidly acquires a pink-red colour. On agitation with chloroform, rubeserine is dissolved out, and colours the chloroform orange-red.

If a drop of a very dilute solution of eserine be placed on a white plate, and brought in contact with a drop of dilute (5 per cent.) solution of caustic alkali, the point of contact will acquire a red colour. On evaporation the liquid becomes yellow, and the residue salmon-pink, and soluble in water with yellow colour.

If a minute quantity of eserine or one of its salts be treated with excess of ammonia, and the liquid heated on the water-bath, it turns in succession pale red, red, yellowish-red, yellow, green, and finally blue. If the liquid be evaporated to dryness, a blue or bluish residue is left. This is soluble in alcohol with blue colour, and, on concentrating the solution, the colouring matter is deposited in long prisms, capable of dyeing silk without a mordant, and staining the skin, &c. The colour dissolves in dilute acetic or other dilute acid with purple-red coloration, and the solution exhibits a strong reddish fluorescence when viewed by reflected light.[1] On evaporation, this liquid leaves a residue which is first green, but changes subsequently to blue, and is then soluble in alcohol, water, or chloroform, but not in ether. From the watery ammoniacal solution, chloroform only partially extracts the blue colouring matter. On treating the above-mentioned blue solutions with sulphuretted hydrogen or other reducing agent, they are first reddened and then decolorised. On heating the decolorised liquid on the water-bath, so as to expel the sulphuretted hydrogen, the blue colour returns.

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[1] The ammonia test for eserine was first described in 1872 by Petit (*Journ. de Pharm.*, [4], xiii. 327), and is official in the French Codex and U.S. Pharmacopœia. In the British Pharmacopœia of 1885, for some unaccountable reason, potash is substituted for ammonia, with the effect of rendering the test worthless, as pointed out by J. C. Umney (*Pharm. Jour.*, [3], xx. 1061) and confirmed by the author.

All the foregoing colour-reactions appear to be due to the formation or decomposition of rubeserine. According to Eber (*Pharm. Zeit.*, 1888, p. 483), rubeserine is not a simple oxidation-product of eserine, a strongly alkaline volatile base being simultaneously formed, which, like rubeserine, has no action on the pupil. Neither rubeserine, the blue compound, nor the volatile base were found by Eber in the urine of an animal to which eserine had been administered; but a base was separated which closely resembled eserine except for the fact that it had no action on the pupil. The same inactive base was separated from certain samples of commercial eserine, and is stated by Eber to be formed by boiling a neutral solution of eserine. Eber believes this base to be an intermediate product in the formation of rubeserine from eserine, and attributes to its presence the very rapid reddening of some samples of commercial eserine. These results are confirmed by Ehrenberg, who finds that if alkalies be allowed to act on eserine in the absence of air and in the cold, a new base is formed, and can be obtained in crystals by means of dry ether. Ehrenberg calls this substance eseroline, and attributes to it the formula $C_{13}H_{18}N_2O$. On exposure to air it rapidly oxidises to rubeserine. It is not yielded by similar treatment of eseridine.

According to Harnach and Wittowski, calabarine is formed by the action of alkalies on eserine.

Eserine is decomposed by boiling with dilute acids.

If a solution of eserine or one of its salts be heated to boiling, and a few drops of strong nitric acid added, an orange-coloured liquid is obtained, which on addition of caustic soda in excess yields an intensely violet solution, becoming wine-red on dilution with water. The violet colour is changed to pale orange by acids, and restored by alkalies (J. E. Saul, *Pharm. Jour.*, [3], xvii. 642; xxiv. 300).

Another form of the nitric acid test has been described by S. J. F. da Silva (*Compt. rend.*, cxvii. 330). A minute fragment of eserine or one of its salts is treated in a porcelain capsule with a drop or two of fuming nitric acid. The clear yellow solution which is obtained changes to orange when heated to 100°, and on evaporation to dryness on the water-bath, while stirring, a green residue is obtained, for which da Silva proposes the name chloreserine. This dissolves in water, or more readily in strong alcohol, with deep green colour, the colouring matter being recovered unchanged on evaporation. The residue dissolves in strong sulphuric acid to form a green, non-fluorescent solution. Ammonia does not affect chloreserine, but if a drop of nitric acid be allowed to fall on the green residue while on the water-bath, it is turned blue in those parts not directly in contact with the acid, and subsequently a reddish-violet solution is formed, which changes after a time to greenish-yellow. This solution, when diluted, is fluorescent, appearing of a blood-red colour by reflected light, and greenish-yellow by transmitted light.

With strong sulphuric acid, solid eserine yields a yellow or brownish coloration, which changes gradually to orange or red. Concentrated hydrochloric acid also produces a pink or reddish colour when added to solid eserine, especially if the mixture be warmed.

Bromine-water, avoiding excess, gives an intense red colour with eserine, both in the solid state and in solution. Bleaching powder solution behaves somewhat similarly, but its action is difficult to control.

Except in very concentrated solution, eserine yields no precipitate with platinic or mercuric chloride, potassium dichromate, or picric acid. Phospho-molybdic acid and iodised potassium iodide precipitate eserine from very dilute solutions. With Mayer's reagent, eserine yields a precipitate of the composition $B,HHgI_3$, melting at 70° C., crystallising from alcohol in small prisms, and soluble in a mixture of alcohol and ether.

According to Eber, auric chloride, potassio-bismuth iodide, and potassio-zinc iodide, used as precipitants of eserine from a solution of the sulphate, give distinct reactions with too minute quantities for recognition by the physiological test.

Eserine derives its chief interest and practical importance from its energetic action on the pupil of the eye, which is powerfully contracted by very minute quantities of the alkaloid. Thus, if 0·010 gramme of eserine be dissolved in 10 c.c. of water, and one or two drops of this 0·1 per cent. solution be introduced into the eye, the pupil will become contracted to the size of a pin's head. The time required for this to occur will be not longer than fifteen minutes, but varies with different persons. Little or no inconvenience is occasioned, and the eye soon returns to its normal condition.

Administered internally, eserine acts as a powerful poison. The principal symptoms are usually severe griping pains, constant vomiting, and contracted pupils. Staggering and purging are also observed in some cases.

Eserine (in the free state, under the name of physostigmine) is official in the British Pharmacopœia, which describes it as occurring in colourless or pinkish crystals. The alkaloid is also official in the French Codex. In the U.S. Pharmacopœia, physostigmine sulphate and salicylate are official. *Physostigmine sulphate*, B_2,H_2SO_4, is described as a white or yellowish-white, micro-crystalline powder, very deliquescent, and gradually reddening on exposure to air and light. It melts at 105°, and dissolves very readily in alcohol and water, forming solutions neutral to litmus. *Physostigmine salicylate*, $B,C_7H_6O_3$, forms colourless or faintly yellowish shining needles, or short columnar crystals, soluble in 150 parts of cold water, or 30 of boiling water. It is also soluble in 12 parts of cold alcohol, and more readily on boiling. The salt melts at about 179°, has a faintly acid reaction to litmus, and acquires a reddish tint on exposure to light and air. *Physostigmine benzoate*, $B,C_7H_6O_2$, is prepared by mixing ethereal solutions of eserine and benzoic acid, and evaporating the ether, when the salt separates in short white prisms, which melt at 115°–116°, are not deliquescent, but dissolve in about four parts of cold water, and are also soluble in alcohol. *Physostigmine hydrobromide*, B,HBr, forms fibrous masses, non-deliquescent, and very soluble in water.

Eseridine is a substance obtained by Eber (*Pharm. Zeit.*, 1888, p. 611) by the action of sulphurous acid on eserine, from which it is said to differ by the elements of water, having the formula $C_{15}H_{23}N_3O_2$. As a reducing agent is used in its preparation this composition is improbable. Eseridine has been prepared in the crystalline state by Boehringer, and has been proposed as a substitute for eserine in therapeutics, on the ground of its milder action. Its advantage over eserine is, however, denied by Schweber, the chief drawback to the use of either alkaloid being the ready susceptibility of the heart to its action.

Eseridine forms small crystals melting at 132°, and evolving alkaline vapours when heated more strongly. The base itself is neutral to litmus.

Eseridine is readily soluble in chloroform, but only sparingly soluble in ether or alcohol.

In its chemical reactions eseridine closely resembles the parent alkaloid, the chief differences being the following:—With ammonia, lime-water, or caustic soda, eseridine becomes gradually yellow and dirty-green, not red like eserine. On agitating a trace of eseridine with a minute particle of potassium iodate and water, a red solution is obtained, from which chloroform extracts a brownish-red colouring matter. In presence of acetic acid, the chloroform becomes violet from separation of iodine.

Calabarine is an alkaloidal substance described by Poehl (*Pharm. Jour. Russland*, xvii. 38), who obtained it from the solution from which eserine had already been extracted by agitation with ether, in which menstruum calabarine is stated to be insoluble, though soluble in water and alcohol. P. MacEwen (*Pharm. Jour.*, [3], xvii. 642) was unable to obtain any evidence of the presence of calabarine in the beans examined by him, and Ehrenberg denies its existence ready-formed in the plant, from which, however, he extracted another alkaloid which he calls *eseramine*. This body crystallises in colourless needles melting at 238°, and apparently having the formula $C_{16}H_{25}N_4O_3$. It is almost without physiological action.

Alkaloids of Gelsemium.

The root-bark of yellow jasmine (*Gelsemium sempervirens*) contains the alkaloids gelsemine and gelseminine in combination with gelsemic acid (page 23).[1] The former alkaloid is crystallisable, and forms crystallisable salts, while gelseminine and its salts are amorphous. Gelsemine is described in Merck's lists under the title of "crystallised gelseminine." This unfortunate misnomer has led to serious confusion between gelsemine and the far more active amorphous base, to which alone the name gelseminine should be applied. The total alkaloids of gelsemium root are stated to range from 0·15 to 0·25 per cent., about three-fourths being gelsemine, though the medicinal activity of the tincture is mainly if not entirely due to the smaller amount of gelseminine.

For the extraction of the mixed alkaloids of gelsemium root, F. A. Thompson (*Pharm. Jour.*, [3], xvii. 806) treats the finely powdered drug with about one-sixth of its weight of slaked lime, and exhausts the mixture with strong alcohol. The percolate is rendered faintly acid with dilute sulphuric acid, the calcium sulphate filtered off, and the filtrate evaporated to a syrup, which is allowed to cool, and water added as long as a precipitate is produced. On standing for twenty-four hours the liquid separates into two strata, the upper of which is chiefly gelsemic acid, and the lower a solution of the sulphates of the alkaloids. This is drawn off, and the gelsemic acid washed with cold water. The solution is concentrated, and agitated with ether to remove a further portion of the gelsemic acid, the last portions being removed by similar treatment with chloroform. The aqueous liquid is then treated cautiously with caustic soda, and the liberated alkaloids extracted by agitation with chloroform. The chloroformic solution is separated, and the alkaloids dissolved out by agitating with water, acidulated with sulphuric acid (not hydrochloric acid). From the dark solution of the sulphates thus obtained, the alkaloids are again liberated by alkali, shaken out with ether, and recovered from the separated ethereal solution by agitation with dilute hydrochloric acid. From this solution the sparingly soluble hydrochloride of gelsemine is deposited on standing, and may be purified by repeated crystallisation from hot alcohol, while the very soluble gelseminine salt remains dissolved.

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[1] According to Ford and Crow (*Pharm. Jour.*, xxiii. 924) the poisonous alkaloid of *Gelsemium elegans*, a species growing in China, is not associated with gelsemic acid, and gives, with sulphuric acid and manganese dioxide, a deep purplish-violet coloration, changing to a rich purple hue. *Gelsemium elegans* has been repeatedly used for criminal purposes in Canton and Hong Kong.

An alternative method is to extract the gelsemium root with a mixture of three measures of alcohol and one of ether, evaporate the solution, take up the residue with water, and precipitate the filtered liquid with lead acetate. The filtrate is freed from excess of lead by sulphuretted hydrogen, and shaken with ether. The aqueous liquid is treated with caustic alkali, and the precipitated alkaloids extracted by agitation with ether.

GELSEMINE[1] is a base of somewhat uncertain formula, but probably contains $C_4H_{28}N_2O_4$. Sonnenschein adopts the formula $C_{22}H_{38}N_2O_4$, while recent investigations by L. Spiegel point to $C_{22}H_{26}N_2O_3$ or $C_{24}H_{28}N_2O_4$. Cushny suggests the improbable formula, $C_{49}H_{63}N_5O_{14}$, and F. A. Thompson that of $C_{54}H_{60}N_4O_{12}$.

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[1] For the extraction of gelsemine, Wormley recommends that the finely-powdered root should be exhausted with a mixture of equal measures of rectified spirit and water, and the clear liquid concentrated to a small bulk, allowed to stand for some time, and filtered. The filtrate is rendered slightly alkaline with ammonia, and agitated several times with ether. The ethereal solution, which contains both the gelsemine and the gelsemic acid, is treated with a slight excess of hydrochloric acid, added drop by drop, and allowed to stand for some hours. The alkaloid is precipitated as a more or less granular or crystalline hydrochloride, which adheres to the sides of the vessel. The ether is poured off, the precipitate washed with fresh ether, dissolved in a minimum of water, and the filtered solution treated with a slight excess of ammonia, which precipitates the gelsemine as a white curdy mass, which is rapidly filtered off, washed with a little cold water, and dried. On allowing the ammoniacal filtrate to evaporate spontaneously, prismatic crystals of gelsemine hydrochloride are deposited. The ethereal solution from which the gelsemine hydrochloride was precipitated on evaporation leaves crystalline tufts of gelsemic acid, which may be washed with a little cold absolute alcohol, and recrystallised from boiling alcohol. From the dried root of *Gelsemium*, Wormley obtained, on an average, about 0·25 per cent. of gelsemine and 0·5 of gelsemic acid. He believes these principles to exist solely in the root-bark, and to be entirely absent from the woody portion.

Gelsemine usually forms a white curdy mass, but is obtainable, with some difficulty, in crystals. It melts at 45° to a colourless liquid, which, on cooling, solidifies to a transparent vitreous mass. Gelsemine is sparingly soluble in water (1 : 644), more readily in alcohol, and very easily in ether and chloroform. It has a strong alkaline reaction, a persistent and very bitter taste, dilates the pupil, and is poisonous.

Gelsemine forms a series of well-defined and crystallisable salts. B, HCl forms sparingly soluble prisms which crystallise concentrically, and darken at 330° without melting. B_2,H_2PtCl_6 and B, $HAuCl_4$ are yellow precipitates, soluble in hot water, and deposited in crystals on cooling. The hydrobromide and hydriodide are crystallisable and unstable; the nitrate forms octahedra, melting at 188° with decomposition.

Gelsemine yields some interesting colour-reactions. Strong nitric acid dissolves the pure alkaloid with little or no colour, but on allowing the liquid to evaporate spontaneously in porcelain, a permanent bluish-green colour is obtained, even when only a very minute trace of gelsemine is present. As usually obtained, gelsemine residues yield with nitric acid yellowish or brownish-green colorations, rapidly changing to deep green.

Pure gelsemine dissolves in strong sulphuric acid without coloration, even on warming; but if not perfectly pure, a more or less reddish or brownish colour is obtained, which gradually becomes pinkish, and on heating acquires a chocolate or purple tint.

When solid gelsemine, or one of its salts, is treated with strong sulphuric acid and an oxidising agent (*e.g.*, potassium bichromate or ferricyanide, manganese dioxide, &c.), in the manner employed in testing for strychnine, a fine reddish-purple or cherry-red coloration is produced, rapidly changing to a bluish-green or blue tint. Wormley states that 0·0001 grain will respond to this test, and that even $\frac{1}{10}$th of this quantity may produce the reddish-purple coloration. The foregoing colour-reaction fails if the mixture of alkaloid with sulphuric acid be heated before adding the oxidising agent; but Wormley states that the alkaloid is not destroyed thereby, since it may be recovered by rendering the solution alkaline, and extracting with ether. The behaviour of gelsemine with the oxidising mixture somewhat resembles that of strychnine, but differs therefrom in the order in which the tints appear (compare Strychnine, Part ii. page 368 *et seq.*).[1]

Gelsemine gives precipitates with most of the general reagents for alkaloids, but they are not very characteristic or highly insoluble. Iodised potassium iodide gives a brown amorphous precipitate in a solution of 1 in 10,000.

In frogs, gelsemine produces tetanic convulsions, followed by paralysis of the extremities of the motor nerves. On rabbits its poisonous effects are not marked.

GELSEMININE (Merck's "amorphous gelseminine") is an alkaloid of doubtful formula occurring with gelsemine. It is described by L. Spiegel (*Ber.*, xxvi. 1054) as a white amorphous powder which softens at 105° and melts at 120°, with partial decomposition.

Gelseminine is intensely bitter, and exhibits a strong alkaline reaction. It is insoluble in water, but soluble in alcohol, ether, and chloroform.

The salts of gelseminine are soluble, amorphous, yellowish bodies.

With diluted nitric acid gelseminine yields brown indefinite compounds, together with an acid of the formula $C_{17}H_{20}N_3O_8$. This compound is deposited from alcohol in pale yellow crystals, which darken without melting when heated to 350°. With strong nitric acid, gelseminine gives a green, and with sulphuric acid a yellow coloration, changing on addition of oxidising agents to violet, and finally to green.

Gelseminine dilates the pupil, and acts as a powerful poison, producing respiratory failure in rabbits as well as in frogs. 0·001 gramme of the alkaloid proved fatal to a rabbit, whereas 0·5 gramme of gelsemine was without effect. It is very probably to the gelseminine (perhaps modified by the co-occurring resinous bodies) that the action of gelsemium tincture is due.

A method of separating gelsemine from gelseminine, for which

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[1] J. B. Nagelvoort finds the distinction between gelsemine and strychnine to be sharpest when the oxidising reagent is a freshly-made mixture of 16 c.c. of strong sulphuric acid with 10 c.c. of water containing in solution 0·020 gramme of potassium permanganate. Strychnine and its salts and solutions, when dropped into this mixture, give an intense *blue* coloration, rapidly changing to purple, and finally a permanent cherry-red; while gelsemine and its preparations never give a blue tint, but always and immediately a cherry-red, which reaction fades to colourless in less than a minute. 0·002 gramme of a gelsemine or gelseminine salt, dissolved in 2 c.c. of water, and added to 3 c.c. of the oxidising mixture, gives the reaction perfectly.

F. A. Thompson claims priority (*Pharm. Jour.*, [3], xvii. 806), is based on the solubility of the hydrochloride of the latter base in an equal weight of water, whereas gelsemine hydrochloride is but sparingly soluble (1 : 39).

GELSEMIC ACID, $C_{30}H_{34}O_{19}+2H_2O$, occurs in combination with gelsemine in yellow jasmine. It crystallises in groups or tufts of prisms, or in minute scales and plates. The melting-point is stated at 163° C. and at 197°. It volatilises unchanged, except for loss of water, and condenses in crystals of characteristic microscopic appearance. Gelsemic acid is colourless, odourless, and nearly tasteless. It is not decidedly acid to litmus. It is poisonous in large doses. Gelsemic acid is soluble in hot water, but is nearly all deposited on cooling, except in the presence of gelsemine or other foreign matters, in which case it is much more soluble. It is readily soluble in alcohol, and also in warm carbon disulphide, ether, and chloroform, but insoluble in hydrochloric acid (distinction from æsculin).

In the fixed alkalies and in ammonia gelsemic acid dissolves to a liquid having an intense yellow colour by transmitted light, but which by reflected light exhibits a rather greenish fluorescence, perceptible even in extremely dilute solutions (1 : 100,000). The fluorescence is readily destroyed by free acids, which fact distinguishes it from the fluorescence produced by quinine.

Nitric acid dissolves gelsemic acid with yellow or orange colour, which is changed by excess of ammonia to a permanent blood-red tint. The reaction is very delicate, but is also produced by æsculin.[1]

Gelsemic acid dissolves slowly in strong sulphuric acid with yellow or reddish colour, not materially changed by warming, if the pure substance be used. On adding potassium bichromate, reduction, with green coloration, ensues. If a drop of ammonia be added to a drop of solution of gelsemic acid in strong sulphuric acid, placed on a slip of glass; gelsemic acid immediately separates at the junction of the two drops in crystalline needles of a characteristic microscopic appearance. Wormley describes this reaction of gelsemic acid as one of the most delicate and characteristic known, and as not readily interfered with by the presence of foreign matter, with the additional advantage of not being simulated by æsculin.

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[1] ÆSCULIN, $(C_{15}H_{16}O_9)_2+3H_2O$, presents a close resemblance to gelsemic acid in many of its reactions, and some observers have regarded the two bodies as identical; but T. G. Wormley, to whom much of the existing knowledge of the subject is due, has pointed out the following similarities and distinctions between them:—Æsculin resembles gelsemic acid in the blue fluorescence of its alkaline solutions, and in giving a red coloration with nitric acid and ammonia; and they are not sharply distinguished by their behaviour with sulphuric acid. Æsculin yields no crystals when its solution in sulphuric acid is treated with ammonia, but is readily soluble in hydrochloric acid, which does not dissolve or act on gelsemic acid even at 100° C.

Unlike gelsemic acid, æsculin exhibits a distinct acid reaction to litmus. It loses two molecules of water at 110°, and the third near its melting point, 197°.

Gelsemic acid is sparingly soluble in chloroform (1 : 230 at 0° C.), but æsculin is quite insoluble either in cold or warm chloroform.

The acid properties of gelsemic acid are very feebly marked, and but few definite metallic salts are obtainable, the precipitates produced by adding metallic solutions to a solution of gelsemic acid in a minimum quantity of an alkali being usually indefinite mixtures of free gelsemic acid and the metallic oxide, or merely due to the action of the acid as a reducing agent. In ammonia, gelsemic acid dissolves apparently without forming a compound, for the acid is extracted unchanged on agitating the alkaline liquid with ether, or by mere evaporation is obtained free and in a crystalline form.

Gelsemic acid is not known to possess any active medicinal properties, but its reactions, and especially its fluorescence in alkaline solutions, are of service as a test for preparations of gelsemium.

Alkaloids of Ipecacuanha.[1]

The roots of *Cephaëlis ipecacuanha* and *C. acuminata*[2] have been long known to contain an alkaloidal principle having violent emetic properties, and hence called emetine. Owing to the amorphous character of the alkaloid and its salts, as ordinarily prepared, and their unstable character, the preparation of the pure base presents great difficulties, and hence very discordant formulæ and characters have been assigned to the alkaloid. Pure emetine appears to have been first prepared by Glenard, in 1876 (*Ann. Chimie & Physique*, [5], viii. 233), who ascribed to it the formula $C_{15}H_{22}NO_2$. In 1893, Paul and Cownley (*Pharm. Jour.*, [3], xxiv. 61) announced the isolation of a second alkaloid from ipecacuanha, which alkaloid they called cephaëline, and this they have since proved to co-exist with emetine in the root from various sources. The following description of the alkaloids of ipecacuanha is taken from subsequent papers by Paul and Cownley, which also contain an interesting historical account of previous researches (*Pharm. Jour.*, [3], xxv. 111, 373, 690).

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[1] The author is indebted to Mr R. A. Cripps for the perusal and correction of this section.

[2] The commercial varieties of ipecacuanha have been recently described by E. M. Holmes (*Year-Book Pharm.*, 1893).

In the examination of Brazilian ipecacuanha (*C. ipecacuanha*), a quantity of the drug was extracted with alcohol, the liquor mixed with basic lead acetate until no further precipitate was formed, the filtered liquid evaporated to dryness, and the residue dissolved in weak acid. The filtered liquid was mixed with ether, ammonia added in slight excess, and the whole shaken. The ethereal solution of the alkaloids was separated, shaken with dilute sulphuric acid, and the acid liquid treated with excess of caustic soda, which precipitated the emetine, while retaining the cephaëline in solution. The precipitate of crude emetine thus obtained, after washing and drying, amounted to 1·34 per cent. of the root used. To purify the emetine it was dissolved in dilute acid, and the solution shaken with caustic soda in presence of ether, this operation being repeated until the cephaëline had been completely separated. The insoluble emetine was then converted into hydrochloride, the salt recrystallised from water, and the base finally precipitated by ammonia. The caustic alkaline solution resulting from the above treatment was neutralised, ammonia added, and the cephaëline extracted by agitation with ether, which on evaporation left 0·6 per cent. on the weight of the root.

In the examination of New Granada ipecacuanha (*C. acuminata*), the powdered drug was mixed with lime and extracted with amylic alcohol, the subsequent separation of the extracted bases being effected as in the previous case.

Both emetine and cephaëline are present in Brazilian ipecacuanha, and also in that from Carthagena, New Granada. In the drug from the latter source, cephaëline appears generally to predominate over emetine, and probably that is also the case in the stalky portion of the Brazilian drug.

The following are the characters ascribed by Paul and Cownley to emetine and cephaëline prepared in the foregoing manner:—

EMETINE, $C_{15}H_{22}NO_2$,[1] is a nearly colourless base, and apparently uncrystallisable. It melts at about 68° C., and rapidly acquires a yellowish colour on exposure to light. It is only slightly soluble in water, but dissolves readily in alcohol, ether, chloroform, and benzene, though only very sparingly soluble in petroleum-spirit, even when hot, as it melts to a resinous mass not readily acted on. The solutions become coloured on exposure to light, and give a reddish deposit. On evaporation of any of these solutions, the emetine is left as a transparent varnish, which is strongly alkaline to litmus and neutralises acids completely.

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[1] H. Kunz-Krause (*Arch. Pharm.*, ccxxxii. 466) has pointed out that the formulæ attributed by Paul and Cownley to emetine and cephaëline contain an uneven number of valencies, and hence are *primâ facie* improbable. He maintains the accuracy of his own formula for emetine, $C_{30}H_{40}N_2O_5$, which he believes to contain four methoxyl-groups, and almost certainly one hydroxyl-group, $C_{26}H_{27}N_2(OMe)_4.OH$. It is probable that Paul and Cownley's formulæ for emetine and cephaeline should be doubled. Analyses of platinum and gold salts, prepared by R. A. Cripps (*Pharm. Jour.*, [4], i. 160) agree closely with Paul and Cownley's analysis.

When precipitated from the solution of one of its salts by caustic alkali, emetine is insoluble in excess of the reagents.

The *sulphate*, *acetate*, and *oxalate* of emetine are very soluble in water and alcohol, and apparently uncrystallisable. The *hydrochloride*, B,HCl, may be obtained in a crystalline condition by evaporating its aqueous solution slowly, or by adding ether to its alcoholic solution. It is nearly insoluble in excess of hydrochloric acid, which converts the base into a mass of silky crystals of B,HCl. A 5 per cent. solution of the hydrochloride, mixed with potassium bromide or iodide, gives a dense precipitate, which dissolves on addition of alcohol, the solution yielding tufts of silky needles of the *hydrobromide* or *hydriodide* of the base when slowly evaporated.

Emetine nitrate is very sparingly soluble in water, and separates as a resinous mass on adding potassium nitrate to a 5 per cent. solution of the hydrochloride. It dissolves more readily in alcohol, and by gradually adding ether to the solution the salt separates in crystalline tufts. B_2,H_2PtCl_6 is a buff-coloured, amorphous precipitate, almost insoluble in water or alcohol.

CEPHAËLINE, $C_{14}H_{20}NO_2$, appears to be the lower homologue of emetine. When precipitated from a solution of one of its salts by ammonia, it is colourless, but rapidly becomes yellow on exposure to light. The ammonia precipitate melts at about 102°. By evaporating a solution in alcohol or ether the base is left as a faintly yellow transparent varnish, but in a closed vessel a concentrated ethereal solution gradually deposits bunches of delicate silky needles, which melt in a capillary tube at 96° to 98°, and lose 4·78 per cent. of their weight on exposure in a watch-glass to a temperature of 100° C.

Cephaëline is very much less soluble in ether, but more soluble in petroleum-spirit than emetine, from which it is sharply distinguished by dissolving in solutions of caustic alkalies. With the exception of the *hydrochloride*, which is deposited in transparent rhombic crystals from a solution containing excess of hydrochloric acid, salts of cephaëline are apparently uncrystallisable; but they otherwise much resemble the corresponding salts of emetine. B_2,H_2PtCl_6 is of a more pronounced yellow than the corresponding salt of emetine.

The physiological action of emetine and cephaëline appears to be very similar. Both alkaloids act as emetics in doses of about one-sixth of a grain, causing at the same time a feeling of considerable depression.

Except the foregoing, all descriptions of the characters and reactions of emetine have reference to indefinite mixtures of true emetine with cephaëline, as obtained from ipecacuanha. It is probable that other alkaloids are present in some cases, and such bodies have been actually obtained. Thus Cripps and Whitby (*Year-Book Pharm.*, 1891, p. 390) obtained from ipecacuanha a small quantity of alkaloid, which was extracted by chloroform but not by ether from its alkaline solutions. It gave faintly most of the colour-reactions of emetine, but appeared to contain some other body in addition. Cripps and Whitby also obtained an alkaloid, not extracted from the root by rectified spirit, unless previously set free from its natural combination by means of an alkali, but which could be readily extracted by water or dilute acid. This body gave a brick-red coloration with nitric acid; a brownish-purple with sulpho-molybdic acid, not becoming blue on adding hydrochloric acid; a reddish-brown with hydrochloric acid alone; a faint emetine reaction with chlorinated lime, and a yellow precipitate with Mayer's solution.

Paul and Cownley (*Pharm. Jour.*, [3], xxv. 690) have recently announced the isolation of a third alkaloid from ipecacuanha, not improbably identical with that previously obtained by Cripps and Whitby. It exists in the drug in very small quantity relatively to emetine and cephaëline, has apparently a much higher molecular weight than these alkaloids, and differs from them in being very sparingly soluble in ether. It is soluble in alkaline liquids, and hence remains in the mother-liquor from which emetine and cephaëline have been extracted by agitation with ether. It is dissolved on shaking this liquid with chloroform. As obtained by slow evaporation of its ethereal solution, the new alkaloid is obtained in transparent, lemon-yellow prisms, which melt at 138° C., neutralise acids, and dissolve readily in alcohol and chloroform, to solutions which darken on exposure to light, and deposit a brown substance.

Arndt (*Year-Book Pharm.*, 1889, page 136) obtained from ipecacuanha root from 0·3 to 0·5 per cent. of a volatile ammonium base which yielded trimethylamine when distilled with strong alkali. More recently, Arndt has identified the base as choline, which exists in the root as an insoluble tannate. To this Arndt attributes the high results obtained when the assay of ipecacuanha is conducted by exhausting the root with acid solvents.[1]

For the preparation of the mixed alkaloids (emetine and cephaëline of Paul and Cownley) from ipecacuanha, R. A. Cripps recommends that the powdered root should be exhausted by percolation with cold alcohol (60° O. P.), and the alcoholic liquid distilled under reduced pressure. To the extract so obtained dilute hydrochloric acid is added in slight excess, and the liquid filtered. The filtrate is treated with a solution of sodium carbonate, and the precipitated alkaloids collected on a filter, washed slightly, and dried in the dark over strong sulphuric acid. The product is finely powdered and boiled with ether (spec. grav. 0·720), the ethereal solution filtered, and dry hydrochloric acid gas passed through the filtrate to saturation. The alkaloids are precipitated as hydrochlorides, which may be filtered off, dissolved in water, and the alkaloids recovered by precipitation with sodium carbonate, washed with water, and dried over sulphuric acid.

Thus prepared, the mixed alkaloids form a whitish amorphous powder, which rapidly becomes yellow and then brown on exposure to light, and dissolves in alcohol to a yellowish solution. It is very slightly soluble in cold water (1:1000), but somewhat more readily on heating. Alcohol, methyl alcohol, amyl alcohol, chloroform, ether, and benzene dissolve the alkaloids readily, the last three solvents extracting them from aqueous liquids in presence of ammonia. Petroleum-spirit dissolves only traces of the alkaloids in the cold, but takes up a larger quantity on boiling. The mixed alkaloids are also soluble in carbon disulphide, ethyl acetate, turpentine, &c. The solutions of ipecacuanha alkaloids are optically inactive and strongly alkaline to litmus.

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[1] Arndt consequently recommends (*Apoth. Zeit.*, 1890, 780; *Year-Book Pharm.*, 1891, pp. 56, 164) that 10 grammes of the powdered root should be intimately mixed with 5 grammes of sodium carbonate and 1 of crystallised ferric chloride, and the mixture digested under a reflux condenser for one hour with 100 grammes of 60 per cent. methyl alcohol on the water-bath. The liquid is filtered and evaporated to remove the alcohol and volatilise the choline or its decomposition-products, the residue is taken up with 50 c.c. of very dilute ammonia, and the liquid agitated with 25 c.c. of chloroform. The chloroformic solution is agitated with acidulated water, and the resultant aqueous liquid titrated with Mayer's solution, 1 c.c. of which corresponds to 0·0198 gramme of emetine. From 5 to 5·5 c.c. of the reagent should be required, indicating an alkaloidal strength of at least 0·95 per cent. of emetine. Cripps has repeated these experiments, using 100 grammes of ipecacuanha, but has been unable to confirm Arndt's results. A careful examination of the root for volatile alkaloids proved unsuccessful. Dieterich and C. C. Keller have also pointed out that choline would not be extracted by ether or chloroform from aqueous liquids, and hence, even if present, it would not interfere with the estimation of true alkaloids (*Year-Book Pharm.*, 1891, p. 386).

When heated, the alkaloids fuse to a nearly colourless liquid, which gradually becomes yellow or brown. The melting-point is variously stated from 50° to 74°, and doubtless depends upon the relative proportions of emetine and cephaëline present. The alkaloids of ipecacuanha are very bitter and highly poisonous, being violent emetics and depressants. The medicinal dose ranges from $\frac{1}{100}$ to $\frac{1}{25}$ grain as an expectorant, or $\frac{1}{5}$ to $\frac{2}{5}$ grain as an emetic.

The most characteristic of the reactions of the ipecacuanha alkaloids is that with chlorinated lime. If a drop of solution of bleaching powder be applied to a fragment of the solid substance or to one of its salts, and a drop of acetic acid then added, a very persistent bright orange or lemon-yellow colour is produced. If the solution of bleaching powder be added to a solution of the alkaloids in dilute hydrochloric acid, an orange coloration is produced and a yellow precipitate formed.

R. A. Cripps and Whitby (*Year-Book Pharm.*, 1891, page 390) enumerate the following additional reactions of the ipecacuanha alkaloids soluble in ether. Few of the reactions are characteristic, the best being that with sulphomolybdic acid:—

With the solid alkaloids:—Sulphuric acid, very pale yellow, becoming pale brown when heated to 100° C.; sulphuric acid and sugar, faint pinkish-brown, becoming salmon-pink; sulphomolybdic acid, pale yellowish-pink, becoming greenish, and on adding hydrochloric acid greenish-blue, changing to rose with green at the edges; hydrochloric acid alone, no coloration; nitric acid, pale orange-brown, changing to bright orange-red.

With a solution in dilute hydrochloric acid:—Auric chloride, yellow precipitate, soluble on warming; platinic chloride, pale yellow precipitate soluble in spirit; phospho-tungstic acid, white precipitate; Sonnenschein's solution, yellow; potassio-bismuthic iodide, brilliant orange-red precipitate; potassio-cadmic iodide, white; Mayer's solution, white; and iodised potassium iodide, bright brown.

The alkaloids of ipecacuanha neutralise acids perfectly, and hence their amount can be ascertained by titration with methyl-orange or iodeosin. The plan can be conveniently applied to the alkaloids shaken out by ether or chloroform.

When in a nearly pure state ipecacuanha alkaloids may also be determined with tolerable accuracy by titration with Mayer's solution, if certain precautions be taken.[1]

Ipecacuanha Root.—At present only the root of Brazilian ipecacuanha is official, and as there is some inducement to substitute other varieties, it is important to distinguish these from the official kind. According to H. G. Greenish (*Pharm. Jour.*, [4], i. 137) this may be effected by a very careful microscopic examination of the specimen.[2] Thus, ipecacuanha *root*, whether Brazilian or Carthagena, may be distinguished as such in the form of powder by (a) the shape and size of the starch grains, (b) the absence of vessels, presence of perforated tracheids, (c) the acicular raphides, and (d) the emetine reaction with chlorine. The *stem* may be distinguished from the root (in powder) by (a) the presence of sclerenchymatous cells, (b) of lignified cells of the pith, and (c) of spiral vessels. *Carthagena* ipecacuanha may, in most cases, be distinguished from *Brazilian* by the larger size of its starch grains. In this respect it must be remembered that Carthagena roots with small starch grains occur that are practically indistinguishable, when powdered, from Brazilian roots with large grains. (See also H. G. Greenish, *Pharm. Jour.* [3], xxv. 685.)

The Assay of Ipecacuanha has received much attention, and many different processes have been devised for the purpose. Generally, the aim of investigators seems to have been to obtain the total alkaloids without distinction of emetine from the others. Many of the processes proposed are invalidated by the readiness with which emetine undergoes decomposition. Zinoffski and Dragendorff applied the reaction with Mayer's solution to a hydro-alcoholic extract of the root, after removing alcohol by evaporation, but this process has been shown to give results above the truth.[1] Flückiger, followed by Jones and Ransom, extracted the root with boiling ammoniated chloroform, the last-named dissolving the residue obtained on evaporating this solution in acidulated water and titrating with Mayer's solution. The various processes for the assay of ipecacuanha published prior to 1889 were reviewed by Cripps and Whitby (*Pharm. Jour.*, [3], xix. 721), who recommended the use of acetic ether as a solvent; but Cripps, in a more recent paper (*Pharm. Jour.*, [3], xxv. 1093), gives preference to the following modification of Lyons' process (*Pharm. Jour.*, [3], xvi. 627):—

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[1] To obtain fairly accurate results, the liquid must contain approximately 1 part of the alkaloid in 500 (Jones, *Year-Book Pharm.*, 1886, p. 543), and the addition of Mayer's solution should be continued until a few drops of the filtrate show the same degree of opalescence by addition of more Mayer's solution or of alkaloidal solution.

[2] An examination by Greenish of thirty-two samples of powdered ipecacuanha obtained by purchase from retail pharmacists, showed the absence of any adulterant in the shape of foreign starch or other drugs. Twelve of the thirty-two samples proved to be the Carthagena (New Granada) root, and possibly a few of the twenty classed as Brazilian may have been Carthagena or of mixed origin. Only one of the samples could claim to be really good Brazilian root free from stem, although this is an impurity which can be readily removed from the commercial drug by picking. Of the samples examined, Greenish classes 22 per cent. as good Brazilian, 31 as medium, 10 as bad, and 37 per cent. as Carthagenian. Ranwez and Campin (*Annal. de Pharm.*, i. 114, 238) classified the ipecacuanha of Belgian commerce (Louvain) as follows:—Normal Brazilian, 15 per cent.; too woody, 40; Carthagena (wholly or partly), 15; and false cultivated root (wholly or partly), 30 per cent. The last description is probably intended to apply to the drug offered at the London sales as "East Indian root," probably the rhizome of *Cryptocoryne spiralis*. This has the characteristics of a monocotyledonous rhizome, does not possess the odour of true ipecacuanha, and contains no emetine. The external appearance, microscopic characters, and chemical reactions render it easy to distinguish the drug from true ipecacuanha root.

Place in a small flask (capacity about 50 c.c.) 2·5 grammes of ipecacuanha in fine powder, and weigh the flask, cork, and contents. Nearly fill the flask with a mixture of ether (sp. gr. 0·720) 250 parts, ammonia 10 parts, and alcohol 20 parts, and set aside, shaking occasionally, for twenty-four hours. Now weigh the flask and its contents before removing the cork, decant rapidly as much as possible of the clear liquid, cork the flask, and reweigh. The alkaloids may now be separated from the ethereal solution by repeated agitation with dilute acid, and again washing out from the acid liquid by ether, followed by chloroform, after rendering alkaline with ammonia or sodium carbonate. The alkaloidal solution is evaporated in a current of air, and the residue dried at a temperature not exceeding 60° C., weighed, and finally dissolved in 5 c.c. of $\frac{N}{20}$ HCl and titrated back with $\frac{N}{100}$ NaOH, using iodeosin as an indicator. 1 c.c. = 0·00254 gramme emetine (or 0·00248 gramme, if Paul's formula be taken). The calculations need no explanation, but may be much simplified and several weighings avoided if the extraction be carried out in a small cylindrical percolator, the diameter of which (for 2·5 grammes) should be 11 to 12 mm.

C. C. Keller (*Year-Book Pharm.*, 1894, page 127) has described the following method of assaying ipecacuanha. It has the merits of being tolerably simple, and of yielding results which agree fairly with those obtained by Lyons' process. A weight of

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[1] See *Pharm. Jour.*, [3], xix. 723.

12 grammes of the air-dry drug is shaken in a dry bottle for five minutes with 90 grammes of ether and 30 of chloroform. 10 c.c. of ammonia is next added, and after half an hour 10 c.c. of water. 100 c.c. measure of the clear liquid is then poured off, and shaken in a separator with 25 c.c. of 1 per cent. hydrochloric acid.[1] The acid liquid is tapped off, and the shaking repeated with 15 c.c. and with 10 c.c. respectively of the same dilute acid. The acid liquid is then made alkaline with ammonia, and agitated twice with a mixture of 3 parts of chloroform with 2 of ether. The solvent is then tapped off, evaporated, and the alkaloidal residue weighed or titrated with decinormal hydrochloric acid. Instead of weighing the residue, it is in some respects preferable to titrate it with standard hydrochloric acid. As an indicator methyl-orange may be used, but the end-reaction is not very sharply marked (T. P. Blunt, *Pharm. Jour.*, [3], xx. 809). Logwood tincture is preferable. It is a good plan to dissolve the alkaloids in a moderate excess of decinormal hydrochloric acid, and titrate back with $\frac{N}{20}$ caustic soda. Keller states that 1 c.c. of decinormal hydrochloric acid neutralises 0·0254 gramme of emetine, which it would if the formula of neutral emetine hydrochloride were $C_{30}H_{40}N_2O_5,2HCl$, as contended by Kunz-Krause. If $C_{15}H_{22}NO_2$, the formula of Paul and Cownley, be adopted as correct, the weight of emetime combining with 1 c.c. of $\frac{N}{10}$ acid will be 0·0248 gramme; or 0·0241 gramme of a mixture of equivalent proportions of emetine with cephaëline.

The proportion of alkaloids present in ipecacuanha-root is subject to much variation, and owing to the variety of methods employed the published results show still wider discrepancies.

The following table, expanded from one by R. A. Cripps (*Pharm. Jour.*, [3], xxv. 1093), shows the proportions of mixed alkaloids found by various observers in commercial ipecacuanha, together with the outline of the methods employed for its determination.

De-emetinised Ipecacuanha has been found by Kanthack and Caddy (*Practitioner*, June 1893, p. 411; *Pharm. Jour.*, [3], xxiii. 990) to be of great value in the treatment of dysentery. They found the antidysenteric value to be in direct proportion to the amount of alcohol-soluble substances present, provided that the emetine had been completely removed, or existed only in minute quantity. As a fact, the proportion of emetine in the

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[1] It is not stated by Keller what proportion the 100 c.c. measure of liquid removed bears to the total upper layer. It would be preferable to operate upon the whole of the ether-chloroform layer, except for the difficulty of accurately separating it from the semi-solid magnia.

Observer.	*Reference.*	*Variety of Drug.*	*No. of Samples examined.*	*Method Employed.*	*Alkaloids found per cent.*
Stewart, ...	*Y. B. Pharm.*, 1877, p. 263.	...	8	Dragendorff's (titration by Mayer's solution).	1·84
Ransom, ...	*Y. B. Pharm.*, 1887, p 450.	Brazilian.	10	Modified Flückiger (hot ammoniacal chloroform).	1·66
Do.	Do.	E. Indian.	1	Do.	1·70
Lyons, ...	*Pharm. Jour.*, [3], xvii. 627.	Brazilian.	48	Dragendorff's (titration by Mayer's solution).	Near 2·60 (5 under 2·0 p. c.) (10 over 3·0 p. c.)
Snow, ...	*Amer. Drug.*, January 1886.	,,	5	Precipitation by platinic chloride.	2·84
Beck, ...	*Y. B. Pharm.*, 1892, p. 133.	,,	Not stated.	Extraction by chloroform and alcohol.	3·20
Hooper, ...	*Pharm. Jour.*, [3], xxii. 591.	E. Indian root.	1	Extraction by alcohol and titration by Mayer.	1·79
Do.	Do.	,, stem.	1	Do.	1·13
Kottmayer, . . .	*Pharm. Post*, 1892, pp. 913, 935.	Brazilian.	Not stated.	Extraction by acidulated alcohol, purification by lead acetate and lime, and solution in chloroform.	2·32
Do.	Do.	E. Indian.	,, ,,		2·26
Do.	Do.	Carthagena.	,, ,,		1·81
Cæsar & Loretz, .	*Apoth. Zeit.*, vii. 464	Brazilian.	3	Not stated.	1·05
Do.	*Pharm. Jour.*, [3], xxiii. 267.	E. Indian.	1	,, ,,	·54
Do.	Do.	Carthagena.	3	,, ,,	1·38
Keller, ...	*Pharm. Zeit.*, xxxviii. 23.	Brazilian.	7	Extraction by ammoniacal ether and chloroform, separation by acid, liberation by ammonia, and titration.	2·58
Do.	Do.	Carthagena.	3		2·18
Attfield, ...	*Pharm Jour.*, [3], xxiv. 48.	Brazilian root.	2	Extraction by cold ammoniacal chloroform, followed by hot ammoniacal chloroform, and separation.	2·01
Do.	Do.	,, stem.	2		1·68
Paul & Cownley, .	*Pharm. Jour.*, [3], xxiv. 62.	,, root.	8	Not stated.	2·11
Do.	Do.	,, stem.	3	,, ,,	1·25
Do.	Do.	Carthagena.	Not stated.	,, ,,	About 2·0
Arndt, ...	*Apoth. Zeit.*, 1890, p. 781.	...	...	...	0·6 to 1·1
Cripps & Whitby, .	*Pharm. Jour.*, [3], xix. 724.	...	12	Modified Flückiger (hot ammoniacal chloroform).	1·21
Do.	Do.	...	12	Acetic ether extraction.	1·97
R. A. Cripps, . . .	*Pharm. Jour.*, [3], xxv 1094.	Brazilian.	61	By acetic ether or Lyons' process modified.	2·24
Do.	Do.	,, stem.	1		1·70
Do.	Do.	Carthagena.	5		1·81

so-called de-emetinised root ranged from traces to 1·2 per cent., while the yield of alcoholic extract was from 2·5 to 11·3 per cent. The sample which gave the best results was prepared by Merck, and showed on analysis only traces of emetine, with 10·3 per cent. of alcoholic extractive. B. H. Paul found nearly 0·5 per cent. of emetine in de-emetinised ipecacuanha from the same source (*Pharm. Jour.*, [3], xxiv. 212). The nature of the anti-dysenteric principle in ipecacuanha has not been definitely ascertained.

The Ipecacuanhas of English Commerce form the subject of an interesting paper by E. J. Holmes (*Pharm. Jour.*, [3], xxiv. 209. See also H. G. Greenish, *ibid.*, pp. 383, 391; xxv. 689; J. Moeller, xxiv. p. 1008, and C. Hartwich, p. 1088). Spurious ipecacuanhas have been described by T. H. Wardleworth (*Pharm. Jour.*, [3], xxiii. 250), R. A. Cripps (*ibid.*, xxiv. 399), Ranwez and Campion (*Ann. de Pharm.*, i. 238), and others.

Alkaloids of Pomegranate.

The bark of the pomegranate (*Punica granatum*) contains several alkaloids, which are liquid at the ordinary temperature.

PELLETIERINE, $C_8H_{15}NO$, is a liquid of a peculiar odour, and a density of 0·988 at 0° C. It boils and distils at 195°, and darkens and resinifies on exposure to air. Pelletierine is dextrorotatory, soluble in 20 parts of cold water, and readily soluble in alcohol, ether, and chloroform. The alkaloid is removed by chloroform from alkaline solutions, but not from acid or bicarbonate aqueous solutions. The solutions have an alkaline reaction. Pelletierine forms a series of crystallisable salts.

Pelletierine sulphate forms minute, white, acicular crystals, which are freely soluble in water. It is employed in medicine, its chief application being in doses of from 5 to 8 grains as a remedy for tape-worm.

The pelletierine tannate of commerce is a mixture of the tannates of the total alkaloids from pomegranate bark. It forms an amorphous yellowish-grey powder of astringent taste, almost insoluble in water, but soluble in alcohol and in dilute acids.

Pelletierine is precipitated from the solutions of its salts by caustic alkalies, but not by alkaline bicarbonates. It is not precipitated by platinic chloride, but yields precipitates with most other general reagents for alkaloids.

Pelletierine is associated in pomegranate bark with the closely analogous liquid alkaloids, *isopelletierine*, $C_8H_{15}NO$, and *methyl-pelletierine*, $C_9H_{17}NO$; and with the solid alkaloid *pseudo-*

pelletierine, $C_9H_{15}NO$,[1] forming crystals melting at 46° C. (See Tanret, *Bull. Soc. Chim.*, xxxii. 464 ; xxxvi. 256.)

Alkaloids of Jaborandi.

The leaves of jaborandi, *Pilocarpus pennafoliatus*, and other species of the same family contain an interesting alkaloid called pilocarpine.[2]

PILOCARPINE, $C_{11}H_{16}N_2O_2$, forms, with some difficulty, crystals which are extremely hygroscopic. On heating to about 150°, the alkaloid decomposes into jaborine, $C_{22}H_{32}N_4O_4$, pilocarpidine, $C_{10}H_{14}N_2O_2$, and jaboric acid, $C_{19}H_{25}N_2O_5$. The two first bodies, which are well-defined bases, are said to accompany pilocarpine in jaborandi, but it appears more probable that they are decomposition-products, formed during the extraction of the parent alkaloid. Pilocarpine is easily soluble in water, alcohol, ether, benzene, and chloroform. The solutions are dextro-rotatory: $[\alpha]_D = +101{\cdot}6°$. Pilocarpine possesses both acid and basic functions. With acids it reacts as a monacidic base, forming a series of crystallisable salts. It dissolves in solutions of caustic alkalies forming uncrystallisable compounds, soluble in water and alcohol,

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[1] Ciamician and Silber (*Berichte*, xxvi. 2738) propose to substitute the name "*granatonine*" for pseudo-pelletierine, so as to allow of a more simple nomenclature for its derivatives. The base is closely related to tropine, and yields a series of corresponding derivatives, as is shown by the following table:—

Granotonine (pseudo-pelletierine),	$C_9H_{15}NO$.		
Granatoline,	$C_9H_{17}NO$.	Tropine,	$C_8H_{15}NO$.
Granatenine,	$C_9H_{15}N$.	Tropidine,	$C_8H_{13}N$.
Granatanine,	$C_9H_{17}N$.	Hydrotropidine,	$C_8H_{15}N$.
Non-granatanine,	$C_9H_{15}N$.	Non-hydrotropidine,	$C_7H_{13}N$.

[2] For the extraction of pilocarpine, jaborandi leaves should be extracted with rectified spirit to which 1 per cent. of strong ammonia has been added. The spirituous solution is neutralised with tartaric acid, and the alcohol distilled off. The residue is treated with ammoniacal alcohol, the filtered liquid distilled, and the residual liquid shaken with chloroform. The impure pilocarpine left on evaporating the chloroform is converted into the nitrate, or the chloroformic solution of the alkaloid is at once shaken with a slight excess of nitric acid, and the resultant nitrate recrystallised from boiling alcohol. From the salt thus purified the alkaloid may be liberated by ammonia, and extracted by agitation with chloroform. The preparation and characters of the tincture of jaborandi, which is official in the British Pharmacopœia, has formed the subject of an able paper by Farr and Wright (*Pharm. Jour.*, [3], xxii. 1).

and decomposed by carbon dioxide with liberation of pilocarpine.[1] The silver and copper salts are only sparingly soluble. They may be regarded as the salts of the unknown pilocarpic acid, of which pilocarpine is the anhydride.

Solutions of pilocarpine are neutral to litmus, but exhibit a distinctly alkaline reaction to tincture of cochineal.

The salts of pilocarpine are now much used in medicine. The nitrate is official in the pharmacopœias of Britain and Spain, and the hydrochloride in those of the United States, Germany, Austria, Hungary, Holland, and Belgium.

Pilocarpine hydrochloride, B,HCl, forms crystalline laminæ, melting at 197°, and easily soluble in water and alcohol, forming solutions having a somewhat bitter taste, and faintly acid reaction to litmus. B,HNO_3 forms acicular crystals or a white crystalline powder, soluble in about 9 parts of cold water, slightly soluble in cold, but freely in hot rectified spirit.

Pilocarpine and its salts are poisonous. They increase the secretion of saliva, tears, perspiration, and bronchial sputa; reduce the action of the heart, and cause contraction of the pupil.

The physiological action of pilocarpidine resembles that of pilocarpine; but the action of jaborine is antagonistic, dilating the pupil and otherwise simulating the action of atropine and its allies. Commercial pilocarpine hydrochloride is liable to contain jaborine, in which case it raises the pulse, produces dilation of the pupil, and causes thirst and dry skin.

Pilocarpine and its salts give no colour-reaction with strong sulphuric or nitric acid.[2] With sulphuric acid and potassium dichromate it gives a dark green coloration. When solid pilocarpine or its hydrochloride is triturated with excess (1 : 100) of calomel (Hg_2Cl_2), blackening ensues from reduction of the mercury to the state of metal. Cocaine gives no similar reaction.

Pilocarpine hydrochloride gives with Mayer's reagent an amorphous precipitate, which in the course of twelve hours separates at the bottom of the tube in the form of oily drops (compare Phenocoll hydrochloride, part ii. page 85).

Iodised iodide of potassium gives with excess of pilocarpine a brown precipitate, which often appears under the microscope as brown feathery crystal or serrated saw-like forms.

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[1] Pilocarpine is extracted from its alkaline aqueous solution, by immiscible solvents, and therefore its combinations with alkalies must be of a very unstable character.

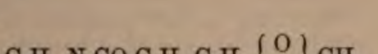
[2] According to Nagelvoort (*Apotheker Zeitung*), the statement of the German and United States Pharmacopœias that pilocarpine hydrochloride dissolves in fuming nitric acid with faint greenish tint is incorrect.

Pilocarpine is precipitated by some other of the general reagents for alkaloids, but gives no reaction with tannin, picric acid, potassium, ferrocyanide, or sodium acetate.

When heated with concentrated hydrochloric acid, pilocarpine is decomposed into methyl alcohol and pilocarpidine, $C_{10}H_{14}N_2O_2$. Strongly heated with water in a sealed tube it splits up into trimethylamine, $(CH_3)_3N$, and pyridine-α-lactic acid, $CH_3.C(C_5H_4N)OH.CO.OH$. The reverse change has been effected by Hardy and Calmels (*Compt. rend.*, cv. 68), and pilocarpine thus synthetically formed.

Alkaloids of Pepper.

PIPERINE. $C_{17}H_{19}NO_3$; or



$C_5H_{10}N.CO.C_4H_4.C_6H_3\left\{\begin{matrix}O\\O\end{matrix}\right\}CH_2$.

Piperine is an alkaloid existing in various plants belonging to the *Piperaceæ*, and is the characteristic principle of both black and long pepper.[1] It also exists in the berries of *Schinus mollis*, a tree belonging to the terebinthaceous order, and has been obtained synthetically by the reaction of its decomposition-products piperidine and piperic acid (chloride).

For the preparation of piperine, white pepper should be exhausted with rectified spirit, the tincture concentrated to an extract, and the extract mixed with caustic potash or soda solution. This dissolves resin, and leaves impure piperine, which is purified by repeated crystallisation from boiling alcohol. Winckler precipitates the alcoholic extract of pepper with basic acetate of lead, exactly precipitates the lead from the filtrate by sulphuric acid (or sulphuretted hydrogen), filters hot, evaporates, treats the residue with water, and boils the undissolved portion with alcohol, from which piperine crystallises on cooling. Another plan is to mix powdered pepper into a paste with slaked lime and water, dry the mixture at 100°, exhaust it with boiling ether, and crystallise from alcohol the piperine left on evaporating the ether. By this process, Cazeneuve and Caillol found in Sumatra pepper an average proportion of 8·10 per cent. of piperine; in white Singapore pepper, 7·15; and in black Singapore pepper, 9·15 per cent.

Piperine forms colourless, nearly tasteless, four-sided, monoclinic prisms. It melts at 128°–129° C., but at a lower temperature if impure. At about 130° it turns brown, and undergoes decomposition.

Piperine is insoluble in cold water, and very slightly soluble in boiling water. It dissolves readily in alcohol, the solution being optically inactive, and destitute of alkaline reaction, but having an extremely pungent taste, like that of pepper. Piperine dissolves sparingly in ether, but with facility in chloroform, benzene, and petroleum spirit.

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[1] According to Dunstan and Carr *Piper ovatum* contains piperovatine, $C_{16}H_{21}NO_2$ (*Jour. Chem. Soc.*, lxvii. 94; *Proc. ibid.*, Nov. 7th, 1895.

Piperine is a feeble base. Its salts are decomposed by excess of water with precipitation of free piperine, and the alkaloid is extracted even from its acidulated solutions by agitation with chloroform, benzene, petroleum spirit, &c. *Piperine hydrochloride* is soluble in alcohol, and on treating the solution with alcoholic mercuric chloride or platinic chloride the corresponding compounds are obtained as crystalline precipitates.[1]

On adding iodised potassium iodide to a hot alcoholic solution of piperine, acidulated with hydrochloric acid, an iodo-compound is formed which separates on cooling in fine steel-blue needles.

Concentrated nitric acid converts piperine into an orange-red resinous substance which is turned blood-red by caustic alkali with formation of piperidine.

With concentrated sulphuric acid piperine instantly gives an orange-red coloration, becoming brown on warming or standing. On addition of water, piperine is precipitated.

When boiled with strong caustic alkali, or heated with soda-lime, piperine undergoes saponification with formation of piperic acid and piperidine, thus:—

$$C_{17}H_{19}NO_3 + H_2O = C_{10}H_{12}O_4 + C_5H_{11}N.$$

Piperidine has the constitution of a hexahydropyridine, and has already been described (Part ii. page 106). Traces of it appear to exist ready-formed in pepper.

Piperic Acid, $C_{12}H_{10}O_4$; or $CH_2{:}O_2{:}C_6H_3.CH_2.CH{:}C{:}CH.COOH$. Piperic acid is the acid product of the saponification of piperine. It is best prepared by boiling a solution of equal weights of piperine and caustic potash in a minimum quantity of strong alcohol for five or six hours under a reflux condenser. The crystalline plates of potassium piperate, which are separated from the brown mother liquor, recrystallised from boiling water, redissolved in boiling water, the solution decolorised by animal charcoal, and treated with hydrochloric acid, when the piperic acid, which separates as a jelly, is washed and recrystallised from alcohol.

Piperic acid crystallises from alcohol in long yellowish needles, often felted together, but when precipitated by adding an acid to the solution of one of its salts it usually separates as a sulphur-yellow jelly, which shrinks on drying. Piperic acid melts at 216°-217°, and at a higher temperature partially sublimes with an odour of coumarin, leaving a brown residue. It is nearly insoluble in water, and requires 270 parts of cold alcohol for solution, but dissolves readily in boiling alcohol. It is sparingly soluble in ether and benzene, and nearly insoluble in petroleum spirit.

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[1] The composition of these salts is said to be respectively $(C_{17}H_{19}NO_3)_2$, $HCl,HgCl_2$, and $(C_{17}H_{19}NO_3)_4,2HCl,PtCl_4$. These formulæ, if correct, suggest that the true formula of piperine is double that usually adopted.

Piperic acid is a feeble acid, but forms a series of crystallisable salts, most of which are but sparingly soluble in water, and insoluble in alcohol.

Concentrated sulphuric acid turns piperic acid blood-red, and subsequently chars it. Nitric acid, even when dilute, converts it into a nitro-derivative of an orange colour, which evolves an odour of coumarin when heated with caustic alkali. When fused with caustic potash, piperic acid yields protocatechuic, acetic, oxalic, and carbonic acids, with hydrogen and secondary products.

COMMERCIAL PEPPER.

The pepper of commerce, when genuine, consists of the immature dried fruit of *Piper nigrum*, a plant indigenous to India, and at present cultivated in the West Indies, various parts of the Malayan Archipelago, and other tropical districts.[1]

Black pepper is composed of the entire berries, with the pulp adhering, gathered before they are quite ripe, and dried in the

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[1] CAYENNE PEPPER is quite distinct in composition, characters, and botanical origin from true pepper. It consists of the ground pods of *Capsicum annuum* or *C. fastigatum*. In the author's personal experience, cayenne pepper has been found adulterated with oxide of iron, common salt, and in one case (1879) with 6 per cent. of red lead. Such sophistications are probably now entirely obsolete.

The following figures are the average of several analyses of commercial cayenne pepper by A. Wynter Blyth:—

Aqueous extract,	32·10	per cent.
Alcoholic extract,	25·79	,,
Benzol extract,	20·00	,,
Ethereal extract,	10·73	,,
Total nitrogen,	2·04	,,
Ash,	5·69	,,

The pungency of cayenne pepper is due entirely to the oil and resin extractable by ether. After exhaustion with this solvent the residue is tasteless, or nearly so.

When added to mustard, gin, &c., cayenne pepper can be detected by the pungent taste of the alcoholic or ethereal extract. A highly characteristic behaviour is the production of intensely irritating vapours on heating the residue. On inhaling these cautiously, the burning sensation produced in the throat and lungs is not to be mistaken.

sun. White pepper consists of the decorticated berries, and hence shows a much smaller proportion of ligneous matter on analysis. To meet the demand for a very light-coloured pepper, the outer layers of the seed are sometimes ground off, and the nearly white kernel alone used. Such pepper contains a large proportion of starch, but is deficient in flavour and pungency.

The commercial value of pepper depends much upon the weight of the peppercorns. The following figures show the weight in grammes of 100 berries of the chief commercial varieties of pepper as observed by A. Wynter Blyth and W. Johnstone.

	A. W. Blyth.	W. Johnstone.
Penang,	6·2496	3·9028
„ white,	...	4·9360
Malabar,	6·0536	...
Sumatra,	5·1476	...
Trang,	4·5736	4·8101
Tellicherry,	4·5076	4·4421
Acheen,	...	5·1976
Alleppy,	...	3·8438
Kampoot,	...	4·4540
Lampong,	...	3·5410
Siam,	...	4·2776
„ white,	...	5·1441
Singapore,	...	4·5338
„ white,	...	4·6936

Blyth remarks that, in the trade, Malabar pepper is generally considered the heaviest, and that his sample of Penang may have been particularly fine. As retailed in a ground state, commercial pepper is invariably blended, a common mixture consisting of equal parts of Malabar, Penang, and Sumatra pepper. Of these, the first, which is the dearest, gives weight, the second strength, and the third colour.

In addition to the usual plant-constituents, including starch, pepper contains an acrid resin, a volatile oil, and the peculiar alkaloid piperine, accompanied in some cases with small quantities of its decomposition-product piperidine.

The *oil* of pepper, which is present to the extent of about 1 per cent., belongs to the terpene class, and has the smell of pepper but only a mild taste.

The *resin* of pepper is said to be dark green, soluble in alcohol, ether, and alkalies, and, in presence of other constituents of pepper, in water also. It has a hot, pungent, peppery taste. From the above description it is very doubtful if the resin has been obtained pure.

Piperine, the characteristic alkaloid of pepper, has already been described. The proportion stated to be present varies within wide limits, but, according to the most reliable observations, lies between 2 and 8 per cent.

Numerous more or less complete analyses of pepper have been published, but for the most part they have little practical value as indications of quality or purity. As the pungency and flavour of pepper depend on the proportion of alkaloid and resin, it is evident that the percentage of these constituents is of the first importance. A. Wynter Blyth (*Foods: Composition and Analysis*) gives the following figures in illustration of the general composition of commercial peppers:—

	Moisture: per cent.	On Moisture-free Pepper; per cent.				
		Total Ash.	Soluble Ash.	Aqueous Extract.	Piperine.	Resin.
Black Peppers—						
Penang, . . .	9·53	4·18	2·21	18·33	5·570	2·08
Tellicherry, . .	12·90	5·77	3·38	16·50	4·675	1·70
Sumatra, . . .	10·10	4·31	2·62	17·59	4·702	1·74
Malabar, . . .	10·54	5·19	3·45	20·37	4·632	1·74
Trang, . . .	11·66	4·77	2·53	18·17	4·600	1·70
White Pepper, . .	10·30	1·12	0·56	..	5·600	2·05
Long Pepper, . .	..	8·30	4·47	16·82	1·800	0·80

The piperine and resin were determined by exhausting the finely-powdered pepper with strong alcohol, evaporating it, and weighing the alcoholic extract, which consists practically of piperine and resin. On treating this with caustic alkali, the resin is dissolved, and the residual piperine is redissolved in strong alcohol, the solution filtered, evaporated, and the residue weighed. The difference between the weight of piperine thus found and the weight of the original alcoholic extract was regarded as resin. An alternative method is to dissolve out the piperine from the alcoholic extract by means of petroleum-ether.

Thos. Stevenson (*Analyst*, xii. 144) in 1887 published the following figures as representing the proportions of piperine and resin in typical samples of commercial air-dried pepper, containing about 14 per cent. of moisture:—

	Piperine.	Resin.
Black pepper, . .	7·14 per cent.	1·44 per cent.
„ (Trang), .	6·62 „	0·82 „
White pepper, . .	6·47 „	0·69 „
Long pepper, . .	4·24 „	1·16 „

In these analyses, a weight of 50 grammes of the ground samples was exhausted with hot methylated spirit at 60° O.P. during several days. The alcoholic liquid was evaporated, and the extracts digested with a cold solution of potash. The piperine thus separated was washed with water, dried at 100° C., and weighed. It was then recrystallised from alcohol, and reweighed in a condition of great purity. The "resin" was obtained by precipitating the alkaline solution with hydrochloric acid, and was no doubt a mixture of resinous and oily bodies.

The following results, published by C. Heisch in 1886, (*Analyst*, xi. 188), afford additional information respecting the variation in composition of commercial peppers:—

	Ash in Moisture-free Pepper.			Per 100 Parts of Ash-free and Moisture-free Pepper.		
	Total.	Sol. in Water.	Insol. in Acid.	Starch.	Alcoholic Extract.	Piperine.
Black Peppers—						
Acheen Penang, . .	8·99	1·54	4·38	48·53	12·26	6·04
Penang, ...	6·446	3·102	·908	51·06	16·20	9·38
Trang, ...	8·35	1·60	3·42	54·06	12·28	4·05
Tellicherry, . . .	5·28	3·34	·04	56·67	12·67	6·88
Tellicherry (brushed), .	6·414	2·739	1·196	55·87	13·62	7·86
Singapore, . . .	5·41	2·07	·82	56·24	12·41	7·14
Light dusty Singapore, .	5·39	2·48	·73	54·93	11·62	6·22
Good black Singapore, .	4·35	2·48	·36	54·54	10·47	6·06
White Peppers—						
Penang, ...	3·779	·618	·357	77·68	9·73	5·54
Singapore, . . .	1·28	·217	·218	76·35	9·49	6·14
Siam, ...	1·807	·254	·688	76·27	9·23	5·13
Fine White (ground), .	1·579	·162	·517	75·31	10·60	4·51
Finest White (ground), .	2·177	·501	·174	84·69	9·53	4·70
Super White (ground), .	1·401	·373	0·00	85·26	9·63	4·50
Long Peppers—						
No. 1, H, . . .	13·48	2·28	5·68	58·98	8·29	1·71
No. 2, T, . . .	11·978	2·374	3·686	46·16	8·52	1·70
Miscellaneous—						
Black Pepper Husks, .	11·902	2·122	3·413	41·71	13·81	4·84
Siftings before grinding,	51·39	1·02	43·90	30·66	7·52	1·15
Poivrette, . . .	3·847	2·88	·38	49·98	...	...

It will be observed that the proportion of "alcoholic extract" obtained by Heisch is in most cases considerably higher than the sum of the resin and piperine recorded by A. Wynter Blyth. Heisch's piperine was obtained crystalline but somewhat dark-coloured. Heisch lays considerable stress on the proportion of *starch*, for the determination of which he boiled the finely-ground pepper for three hours in "10 per cent. hydrochloric acid," and observed the optical rotation of the resulting liquid. The results agreed closely with those obtained by determining the cupric oxide reducing power of the liquid. The gum and other soluble matters were found to possess an optical activity equivalent to about 1 per cent. of starch, while the alcoholic extract was optically inactive. Determinations of cellulose were subsequently made in many of the above samples, with results which are stated on page 54.

In 1884, Lenz (*Zeits. anal. Chem.*, xxiii. 501; abst. *Analyst*, x. 10) also determined the amount of glucose produced by inverting the starch in pepper and its common adulterants. Every one of fourteen samples of pepper of different kinds gave a Fehling reduction equivalent to more than 50 per cent. of the moisture-free organic matter of the pepper, while all the adulterants gave less than 30 per cent., except those of starchy nature, like flour and meal, which, according to Lenz, are not employed in practice in Germany. Lenz regards the determinations of matters soluble in water, alcohol, or ether as practically valueless, and lays stress on the necessity of conducting the inversion exactly in the following manner prescribed by him:—From three to four grammes of the substance to be examined should be treated for three to four hours with 250 c.c. of cold water, the flask being repeatedly shaken. The liquid is then filtered, the residue washed with cold water, and the moist powder washed back into the flask and the liquid diluted to 200 c.c. with water. 25 c.c. of 25 per cent. hydrochloric acid is next added, the flask fitted with a cork and tube one metre long, and immersed in a bath of boiling water for three hours, with occasional shaking. The solution, on cooling, is carefully neutralised with caustic soda, made up to 500 c.c., and titrated with Fehling's solution. When palm-cake was present it was found necessary to add a little zinc chloride to clear the solution.

Rottger has shown that other substances besides starch are inverted in Lenz' process. He finds the sugar-equivalent in black pepper to vary from 57·2 to 60·3 per cent.; in white pepper from 59·6 to 74·4 per cent.; while Lampong pepper gave an equivalent of 41·7 per cent. only. These figures refer in each case to the moisture-free and ash-free samples.

W. Busse (abst. *Analyst*, xx. 180) considers the best criterion of the value of pepper to be found in the determination of the brown colouring substances peculiar to the husk. To isolate these he treats five grammes of the finely powdered and dried pepper with boiling absolute alcohol, grinds up the dried residue with a little water, washes it into a flask with from 50 to 60 c.c. of boiling water, and adds 25 c.c. of a 10 per cent. solution of caustic soda. The flask is warmed on the water-bath for five hours with frequent agitation, after which the contents are nearly neutralised with strong acetic acid, 250 c.c. of water added, and the liquid filtered (with the aid of a filter-pump) after standing at rest for twelve hours. 50 per cent. of the filtrate is next acidulated with acetic acid, and 20 c.c. of a 10 per cent. solution of lead acetate in dilute acetic acid added. The liquid is then diluted with water to 100 c.c., agitated, and filtered. 10 c.c. of the filtrate should be treated with 5 c.c. of dilute sulphuric acid (1 : 3) and 30 c.c. of alcohol, and the resultant lead sulphate filtered off, washed, weighed, and calculated to its equivalent of metal. The amount of lead thus found Busse calls the "lead number" of the sample. The extract from 100 parts of pepper, &c., gives the following percentage of lead when thus treated :—

White pepper,	. . 0·6	to	2·7	per cent. of lead.
Black pepper,	. . 5·4	,,	7·5	,, ,,
Pepper husks,	. . 12·9	,,	15·7	,, ,,
Pepper dust,	. . 10·9	,,	12·2	,, ,,

Adulterations of Pepper.

Pepper is liable to admixture with a variety of *mineral adulterants.* Chalk, sand, clay, barium sulphate, dust, shop-sweepings, and refuse generally are among those occasionally met with.[1]

Such admixtures, when present in considerable proportion, are indicated by the excessive proportion of *ash*, which in genuine white pepper ranges from 0·8 to 2 per cent., and in black pepper from 3·5 to 5·0 per cent.; but when mineral adulterants are added in small quantity, as is now usually the case, the mere percentage of ash does not afford sufficient information.[2] Under these circumstances, a very useful plan is to shake a known weight of the pepper (*i.e.*, 10 grammes) in a tapped separator with chloroform. On allowing the mixture to stand, the mineral adulterants sink to the bottom, together with a small quantity of husk, &c., and can be removed through the tap and further examined.

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[1] At the weekly spice sales in Mincing Lane, pepper adulterated to an enormous extent is sometimes put up to public auction, and readily bought with a full knowledge of its nature. 30 tons of "black pepper dust" have been sold at once under these circumstances at about 2d. per lb., although containing 44·2 per cent. of mineral matter, consisting of stones, lime, and dirt, and an additional 54·8 per cent. of mouldy and unsound pepper leaves, husks, &c.; leaving just 1 per cent. of whole pepper grains! (*Analyst*, viii. 46).

[2] According to a case reported in the *Analyst* (xiv. 59), the Somerset House chemists allow 3½ per cent. of sand in pepper, and an additional 7 per cent. of "mineral matter" is stated to be allowed by "public analysts and writers of authority." Such allowances appear to be quite indefensible.

After evaporating off the chloroform, the deposit may be weighed, observed under the microscope, and treated with solvents for further analysis. This method of operating has the great advantage of distinguishing between added mineral matters and natural ash-constituents.

Bostock Hill (*Analyst*, x. 122) has met with commercial pepper yielding 24·2 per cent. of ash of a reddish colour, which proved on analysis to consist of finely-ground clay or brick-dust.

Pepper-corns themselves are frequently bleached or limed. B. Dyer (*Analyst*, x. 123) has met with samples sold in Mincing Lane as white pepper, consisting in reality of black pepper. This had been skilfully coated with a kind of whitish clay, which all washed off in water, though the peppercorns could be rubbed in the hands without the sophistication being observed.

F. W. Stoddart (*Analyst*, xiv. 37) has described a material in extensive use for the adulteration of pepper, which consists of a finely-ground mixture of rice-starch, barium sulphate, calcium carbonate, and lead chromate. The last ingredient forms about 10 per cent. of the mixture. By the addition of about 5 per cent. of this preparation, the colour of pepper is so improved as to raise its market value very considerably, though as much as 10 per cent. is stated to be sometimes employed. Such pepper is best examined by agitation with chloroform, when the mineral adulterants are separated unchanged.

W. F. K. Stock (*Analyst*, xvi. 224), in 1892, published further information with regard to commercial white pepper. He gives the following figures obtained by the analyses of four samples of whole peppercorns ground in his own laboratory :—

	Tellicherry.	Siam.	Lampong.	Penang.
Ash, ...	1·05 per cent.	1·45 per cent.	2·20 per cent.	2·75 per cent.
Fibre, ...	4·86 ,,	4·43 ,,	4·90 ,,	5·06 ,,
Calcium Carbonate in Pepper, . . .	0·58 ,,	0·62 ,,	0·81 ,,	1·67 ,,
Calcium Carbonate in Ash, . . .	55·20 ,,	42·70 ,,	36·80 ,,	60·70 ,,

Stock obtained the following figures by the analysis of two samples of Tellicherry pepper :—

	Undecorticated.	Decorticated.
Total Ash, ...	4·02 per cent.	1·64 per cent.
Fibre, ...	10·40 ,,	6·80 ,,
Ratio of Lime as $CaCO_3$ to total Ash, . .	27·30 ,,	62·00 ,,

From these figures it appears that the natural calcium compounds of pepper are more abundant in the kernel than in the husk, and that instead of the calcium compounds augmenting directly as the proportion of husk, as asserted by interested parties, they are in inverse proportion.

The foregoing figures cannot be compared with those obtained by the analyses of bleached samples of pepper, because both the calcium compounds and the fibre are greatly affected by the bleaching process. From the fact that by no process of grading can the normal relation of the ash-constituents of the kernel be disturbed, and taking into account also the natural percentage of ash and the normal ratio of lime to total ash, it follows that these determinations give the necessary data for ascertaining any abnormal proportion of calcium salts in white pepper.

Stock gives the following results, showing the amount of ash in 100 samples of white pepper, purchased indiscriminately under the Sale of Food and Drugs Act :—

Between 1 °/o and 1·5 °/o.	Between 1·5 °/o and 2·0 °/o.	Between 2·0 °/o and 2·5 °/o.	Between 2·5 °/o and 3·0 °/o.	Between 3·0 °/o and 3·45 °/o.
26 samples.	37 samples.	18 samples.	9 samples.	10 samples.

The lowest ash found was 0·80 per cent. ; the highest, 3·45 ; and the average, 1·914 per cent.

Stock holds that no sample of white pepper should be considered genuine the ash of which exceeds 3 per cent., and where the proportion of lime, in terms of $CaCO_3$, exceeds 60 per cent. of the total ash. These limits, he considers, allow fair latitude for natural variations and unavoidable impurities.

Long Pepper is the fruit of *Chavica Roxburghii*, and does not consist merely of the berries analogous to the pepper-corns of true pepper plant.[1] As found in commerce, it is always contaminated with from 3 to 7 per cent. of insoluble sand and clay, imbedded in the crevices and irregularities of the fruit. Hence it is difficult, if not impossible, to clean long pepper before grinding, in the manner readily practised with true pepper.

As it is not possible to separate the hard husk and woody centre from the minute berries, ground long pepper contains much larger proportions of woody fibre than are characteristic of (ground) true pepper of the corresponding shade, though not so high a percentage of total cellulose as is contained in the most husky black pepper. These facts are exemplified in the following figures by J. Campbell Brown, obtained by the analysis of samples of long pepper carefully cleaned by hand:—

	A.	B.	C.
Total Ash,	8·91	8·98	9·61
Ash insoluble in hydrochloric acid,	1·2	1·1	1·5
Starch and other matter convertible into sugar,	44·04	49·34	44·61
Albuminous matter soluble in alkali,	15·47	17·42	15·51
Cellulose,	15·70	10·50	10·73
Alcoholic extract,	7·7	7·6	10·5
Ethereal extract,	5·5	4·9	8·6
Total Nitrogen,	2·1	2·0	2·3
×6·25=Albuminoids,	13·13	12·5	14·37

In these analyses it will be observed that the albuminoids, calculated in the usual manner from the total nitrogen, are sensibly less than the amount directly determined, although a notable quantity of the total nitrogen existed in a non-albuminoid form (piperine, &c.).

Long pepper contains a much smaller proportion of piperine than is present in true pepper, and the essential oil has a strong and disagreeable smell.

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[1] According to J. Campbell Brown (*Analyst*, xii. 67), long pepper bears much the same relation to true pepper that wild grass seed would bear to oatmeal. It consists of the small berries with the husks and indurated coverings hardened together and to the central woody stem, much as in pines the seed and coverings are all hardened into one cone. Long pepper is usually derived from wild plants, and is always contaminated with a quantity of dirt, picked up from the soil of the river-banks on which it grows.

Long pepper is legitimately used in pickles, but in the ground state is not a recognised article of trade. Its flavour and its smell on warming preclude its use in an unmixed state, and its unacknowledged addition to true pepper is clearly an adulteration (see *Analyst*, xiv. 107).

According to J. Campbell Brown (*Analyst*, xii. 69) the presence of long pepper in ground pepper may be recognised by the following characters:—

Any considerable proportion of long pepper imparts to the mixture its peculiar slaty *colour*; but this may be prevented to some extent by sifting out much of the darker or husky portions of the long pepper before mixing.

The *odour* of a mixture containing even a moderate proportion of long pepper is unmistakable after some experience. The ethereal extract of the samples yields the characteristic odour of long pepper very plainly when warmed. The *ash* of a sample containing long pepper will be excessive, especially the proportion of ash insoluble in hydrochloric acid. This indication is particularly important when long pepper has been added to white pepper, the natural ash of which is very small. When long pepper, from which the husk particles have been sifted out, is added to white pepper, it invariably introduces its sand with it, as also some spent bleach, if an attempt has been made to bleach it. The *woody fibre* in ground long pepper is always considerable. On spreading out a sample containing long pepper in a smooth thin layer on strong paper, by means of an ivory paper-knife, pieces of fluffy woody fibre will be detected, especially if the smooth thin layer be tapped lightly from below. These particles have a characteristic microscopic appearance. The *starch-granules* of long pepper are much larger than those of true pepper. They have a diameter of about 0·0002 inch, and hence are not much smaller than rice-starch. They appear isolated or loosely aggregated in clusters.

According to A. W. Stokes (*Analyst*, xiv. 82), any admixture of long pepper with true pepper can be detected by placing a small portion of the sample on a slip of glass, adding a drop of glycerin, covering it with thin glass, and observing it under the microscope, using a one-inch power and the Nicol's prisms crossed so as to give a dark field. If ordinary pepper alone be present, the field will be entirely dark, but fragments of long pepper exhibit a ghostly white appearance. Stokes points out that rice exhibits a very similar appearance to long pepper when examined in this manner, a fact which has been known to and utilised by the writer for many years.[1] The starch-granules of long pepper are well-defined and angular, but much larger than those of true pepper, approaching the size of the granules of rice-starch.

Ground *rice* is the most frequent amylaceous adulterant of commercial pepper. The author has met with it to the extent of 50 per cent. It can be recognised under the microscope by the size and polygonal shape of the starch-granules, and by the peculiar appearance already referred to when the sample is mounted in glycerin and examined by polarised light. The proportion of rice mixed with pepper is best inferred from the increased percentage of starch.

Spent ginger has been used as an adulterant of pepper. It may be recognised by the microscopic appearance of its starch.

To facilitate the examination of pepper under the microscope, F. W. Rimmington (*Analyst*, xiv. 82) recommends that the sample should be shaken several times with alcohol and subsequently with water in a test-tube. The residue is allowed to subside, when it usually forms several strata, the uppermost of which is the most interesting. On removing a small portion of this with a pipette, and examining it with a magnifying power of about 250 diameters, every particle will be seen clearly defined, the starch-granules can be easily measured, and any foreign bodies can be recognised.

Dhoura, commonly called great millet, or Turkish millet, is the grain (with an integument, but without the husk) of the cereal plant *Sorghum vulgare*. J. Campbell Brown met with it several times in commercial pepper, and gives the following figures illustrative of the composition of the moisture-free substance (*Analyst*, xii. 90) :—

	No. 1.	No. 2.
Ash, ...	1·31 per cent.	1·69 per cent.
Soluble in 10 per cent. hydrochloric acid, . .	90·70 ,,	87·80 ,,
Starch, ...	75·20 ,,	73·00 ,,
Albuminous matters soluble in caustic alkali, .	6·71 ,,	7·96 ,,
Cellulose, ...	2·56 ,,	4·19 ,,
Alcoholic extract, ...	10·36 ,,	7·96 ,,
Ethereal extract, ...	10·10 ,,	7·30 ,,
Nitrogen, ...	1·82 ,,	1·79 ,,

---

[1] Excessive grinding must be avoided, or the substance will be resolved into the ultimate starch-granules, which do not produce the same optical effect.

The body of sorghum seed is very white, and consists mainly of roundish or irregular starch-granules, varying in size from 0·0001 up to 0·0006 inch in diameter, and showing under polarised light a nearly right-angled cross. Some of these granules have a hilum and star in the centre, somewhat like bean-starch. The seed also contains larger, irregularly-rounded, granules of starch, varying from 0·0005 to 0·0013 inch in diameter, and showing no cross, or only a very faint one, under polarised light.

Poivrette or *Pepperette* is the trade-name of an adulterant of pepper which was employed very extensively a few years since, and the nature of which was first pointed out by J. Campbell Brown. Poivrette consists of finely-ground *olive-stones*, and has the composition indicated by the following figures (*Analyst*, xii. 24):—

	White Pepperette.	Black Pepperette.	Almond Shells.	Olive-Stones.
Ash, ...	1·33	2·47	2·05	1·61
Matters soluble in dilute acid, ...	38·32	34·55	23·53	39·08
Albuminous and other matters soluble in alkali, ...	14·08	17·66	24·79	15·04
Woody fibre, &c., insoluble in acid and alkali, ...	48·48	47·69	51·68	45·38
Starch, ...	None.	None.	None.	None.

White poivrette consists of pale, and possibly partially bleached, olive-stones, while black poivrette contains a little black husk. Although poivrette contains no starch, it reduces Fehling's solution after boiling with dilute acid. The quantity of sugar produced depends on the time of treatment and the strength of the acid used, but is approximately 10 per cent.

Poivrette, as it occurs in commerce, is a buff or cream-coloured powder, and cannot be distinguished by the naked eye or a moderate magnifying power from genuine pepper. Under the microscope, with a $\frac{1}{6}$- or $\frac{1}{8}$-inch objective, it is seen to consist of pale, dense ligneous cells, some entire and marked with linear air-spaces, others torn and indistinct. Some of the cells of poivrette are very characteristic, their appearance somewhat resembling grains of oats. A striking difference between the ligneous cells of poivrette and pepper is perceptible when the object is examined by polarised light, with the Nicol's prisms crossed, but without a selenite plate, when the poivrette cells appear of a bluish-white, and the pepper of a yellowish-white lustre (J. Campbell Brown, *Analyst*, xii. 72).

A simple method of recognising poivrette in pepper is to mix the sample into a paste with dilute caustic alkali, dilute the mixture with much water, and wash the residue by decantation. The particles of poivrette will then appear of a bright yellow colour, while the dark particles are pepper-husk. Bleached husk-cells may somewhat resemble those of poivrette, but are distinguished by their softness and appearance under the microscope.

Poivrette may be recognised in pepper by other colour-reactions. Thus Gillet (*Bul. Soc. Chim.*, i. 173) has proposed the use of a solution of 6·5 gramme of iodine in 120 c.c. of rectified spirit, which colours pepper brown or dark chestnut, while poivrette is said to become bright yellow. Jumeau prefers a solution of 5 grammes of iodine in 100 c.c. of a mixture of ether and alcohol. Chevreau (*Rep. Pharm.*, 1889, p. 203; *Pharm. Jour.*, [3], xx. 64) considers the iodine-reaction unreliable, and utilises the fact that the sclerous elements of vegetable tissues are coloured yellow by acid solutions of aniline, while other tissues are unaffected. To test pepper for poivrette he moistens the sample with a solution of aniline in two to three parts of ordinary acetic acid. When genuine pepper is thus treated, no change is observable by the naked eye, and even under the microscope only a few scattered yellow cells can be seen. But if the sample contain ground olive-stones it assumes a characteristic yellow colour, and under the microscope the stone-cells appear of a pure gamboge-yellow. D. Martelli (abst. *Analyst*, xx. 181) digests 1 gramme of phloroglucinol for two or three days in 50 to 60 c.c. of hydrochloric acid of 1·1 specific gravity. A small quantity (0·5 gramme) of the sample of pepper is covered by this reagent and cautiously heated until fumes of hydrochloric acid begin to come off. Poivrette and similar substances, including the shells of almonds, walnuts, &c., are coloured an intense cherry-red, and may be readily distinguished from the yellow or faintly-brown particles of true pepper. On adding a little water and decanting, any poivrette or other woody tissue is left as a violet-red powder.

Pabst (*Monit. Scient.*, xxxiv. 470; abst. *Jour. Soc. Chem. Ind.*, ix. 770) recommends, for the detection of poivrette, a solution of dimethyl-paraphenylenediamine,[1] which reagent has been applied by Würster to the detection of wood-pulp in paper. In testing pepper, 1 to 2 c.c. of the reagent should be placed in a shallow dish and a pinch of the pepper sprinkled into on the surface. In a few minutes, the particles of olive-stones assume a fine carmine-red colour, while the grains of pepper remain unaltered, or are coloured only a faint pink. If water be next added, the heavy particles of poivrette sink and collect together, when they are easily identified. The colour acquired by olive-stones when thus treated dissolves in the water after a time, and the particles acquire a brownish or blackish colour. Other ligneous tissue, such as ground nut-shells, behaves like poivrette with Pabst's reagent. Thalline sulphate, in freshly-made 0·5 per cent. aqueous solution, may be substituted for dimethyl-phenylenediamine solution in the above test. The particles of poivrette are stained a beautiful orange colour, which, however, takes longer to develop than the red produced by the previous reagent.

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[1] This reagent is prepared as follows:—Ten grammes weight of commercial dimethylaniline is mixed in a porcelain dish with 20 grammes of pure strong hydrochloric acid. 100 grammes of broken ice are next added, and then,

According to C. Neuss (*Pharm. Zeit.*, 1885, p. 30), if powdered pepper be covered with concentrated hydrochloric acid, the particles of true pepper assume an intense yellow colour, so that any admixture can be readily recognised, and approximately determined.

According to C. Girard, if pepper adulterated with poivrette be sprinkled on the surface of a mixture of equal measures of glycerin and water, the pepper will float on the surface, while the olive-stones sink to the bottom of the liquid.

The presence of poivrette in pepper having been recognised by some of the foregoing means, the proportion present may be approximately deduced from the abnormal amounts of ash, fibre, and starch.

H. Rabourdin (*Jour. Pharm.*, [5], ix. 289) has described the following method of determining crude fibre in pepper. It is stated to give results within 2 per cent. of the truth, and is favourably reported on by J. Muter (*Analyst*, ix. 197):—One gramme of the sample, in which poivrette has already been detected by the microscope or colour-tests, is boiled continuously for one hour with 100 c.c. of water and 4 grammes of concentrated sulphuric

little by little, with constant stirring, a solution of 7 grammes of sodium nitrite in 100 c.c. of water. After half an hour, 30 to 40 grammes of hydrochloric acid and 20 grammes of tin-foil are added. The reduction is allowed to go on for an hour, when the tin in solution is precipitated by means of metallic zinc. The decanted and filtered liquid is treated with a slight excess of sodium or potassium carbonate, and the turbidity thus produced is redissolved by the addition of acetic acid. Finally, 10 grammes of sodium bisulphite are added, and the whole is diluted to 2 litres.

acid. The flask should be furnished with a reflux condenser to prevent evaporation, and should be supported by the neck to avoid fracture through the violent bumping that occurs. When cool, the liquid is poured through a plain double filter which has been previously well dried and tared. When the sample contains olive-kernels, they adhere to the sides of the flask in form of reddish, sandy particles, which are never obtained when pure pepper is under treatment. The residue is washed thoroughly with boiling distilled water, dried, and carefully weighed. The yield of residue varies considerably with the kind of pepper employed, but is stated to be nearly constant for the same kind, while there is a large increase in the presence of olive-stones. White pepper yielded 17·5 per cent. of residue ; Malabar, Tellicherry, and Saigon peppers, 30·0 ; Aleppy pepper, 32 ; and the so-called light varieties of pepper, 35 per cent. Pepper refuse, consisting largely of the epidermis, gave 65·5 per cent. ; olive kernels, an average of 74·5 ; and olive husks or shells, 70 per cent.

A. W. Stokes has described and recommended a very similar process (*Analyst*, xii. 147). He boils 1 gramme of the sample of pepper for five minutes with 100 c.c. of distilled water, to swell up and burst the starch-granules. 50 c.c. of water, containing 6 c.c. of strong sulphuric acid, are next added, and the whole boiled for an hour under a reflux condenser. The contents of the flask are then washed into a weighed filter, and washed in succession with hot distilled water, hot alcohol, and ether. The filter and its contents are then dried and weighed,[1] burnt, and the ash deducted from the weight of the residue previously found. Operating in this manner Stokes found :—

	Crude Fibre ; per cent.		
	Highest.	Lowest.	Average.
Black pepper,	26·3	21·0	24·4
White pepper,	13·8	12·7	13·3
Long pepper,	22·3	20·0	21·0
Olive-stones,	64·2	62·2	62·5
Rice,	1·6	0·8	1·0

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[1] A correction should be made for the loss undergone by the filter-paper by the treatment. This is best done by employing a double filter, and using the outer one as a counterpoise.

Stokes' figures are uniformly lower than Rabourdin's, either from the fact that his samples are air-dried and the latter observer's moisture-free, or because he used stronger acid, and subsequently washed with alcohol and ether.

In the foregoing analyses, the residue weighed was not pure cellulose. C. Heisch (*Analyst*, xiii. 149) has recorded a number of determinations of cellulose in pepper and its adulterants, the following being a *résumé* of his results, which refer to ash-free and moisture-free samples (compare page 42):—

Kind of Pepper.	CELLULOSE; per cent.		
	Highest.	Lowest.	Average.
White pepper, ...	6·74	3·44	5·33
Black pepper, ...	27·82	11·58	16·71
Long pepper, ...	12·98	11·42	12·20
White poivrette, ...	...	...	61·94
Black poivrette, ...	...	...	68·80

Alkaloids of Sabadilla (*Cevadilla*).

In 1819, the seeds of *Veratrum sabadilla*,[1] called also *Schœnocaulon officinale* and *Asagræa officinalis*, were found independently by Meissner and Pelletier and by Caventou to contain an alkaloid which was obtained by the former chemists in an amorphous form by boiling the seeds with water acidulated with sulphuric acid, and treating the extract with ammonia in excess.

In 1834, Couerbe isolated from a similar product three distinct substances, of which one was amorphous, but yielded a crystallisable sulphate and hydrochloride. It was readily soluble in alcohol and ether, but insoluble in water. To this alkaloid, Couerbe gave the name of *veratrine*. The second alkaloid, called by him *sabadilline*, was insoluble in ether, but soluble in alcohol and water, and was crystallisable therefrom. The third was also soluble in alcohol and water and insoluble in ether, but was amorphous, and formed non-crystalline salts. It was regarded by Couerbe as the monohydrate of sabadilline.

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[1] The several species of *Veratrum* contain a number of very similar alkaloids, the chemistry and exact relations of some of which are still very obscure. Some of these alkaloids are readily saponifiable, like the aconite alkaloids, which fact adds to the difficulty of their isolation. To increase the confusion, the name "veratrine" has been applied by different observers to several distinct bases, besides indefinite mixtures, and the pharmacopœias of this and other countries describe as "veratrine" the mixed alkaloids obtained from cevadilla seeds. Since cevadine, the principal crystalline alkaloid of cevadilla, is quite different in constitution and properties from jervine, the characteristic alkaloid of white and green hellebore, and the associated alkaloids are also for the most part distinct, it is desirable to consider the alkaloids of sabadilla and the hellebores separately.

In 1855, Merck obtained from commercial veratrine, by evaporation of a solution in diluted alcohol, a very pure substance, which he succeeded in crystallising. This body, which he called *veratrine*, gave upon analysis numbers leading to the formula $C_{32}H_{52}N_2O_8$. Its salts, with the exception of the aurichloride, failed to crystallise.

In 1871, Weigelin separated three alkaloids from sabadilla seeds. The one identical with Merck's base he considered as capable of existing in two separate forms, one soluble and the other insoluble in water. The other two alkaloids were obtained by precipitating the first base by ammonia and shaking the filtered liquid with fusel oil.

In 1879, Alder Wright and Luff (*Journ. Chem. Soc.*, xxxiii. 338) announced the presence in sabadilla of three distinct alkaloids, all of them saponifiable. First, an amorphous base, containing $C_{37}H_{53}NO_{11}$, forming a crystallisable sulphate and hydrochloride, and yielding veratric acid, $C_9H_{10}O_4$, on saponification, from which behaviour they named it *veratrine*. Secondly, the crystallisable alkaloid previously described by Merck, Weigelin, and Schmidt and Köppen, to which they assigned the formula $C_{32}H_{49}NO_9$, and called *cevadine*, because it yielded cevadic acid, $C_5H_8O_2$, on saponification. And thirdly, an amorphous base, *cevadilline* containing $C_{34}H_{53}NO_8$, present in very small quantity, and resembling cevadine in yielding cevadic acid on saponification. This base was insoluble in ether, but differed in other respects from the base previously described by Weigelin under the same name. In 1883, Bosetti concluded that the commercial alkaloid from sabadilla contained two alkaloids which he regarded as isomeric. For the crystalline base (Wright's cevadine) he retained the name *veratrine*, while he called the amorphous base *veratridine*. Ahrens and some other recent investigators also use the name "veratrine" to represent *cevadine*, $C_{32}H_{49}NO_9$.[1]

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[1] Wright and Luff's nomenclature is adopted in Beilstein's *Organische Chemie*, in the *United States Dispensatory*, the *National Dispensatory*, &c.; while Richter's *Organic Chemistry* still applies the name "veratrine' to Merck's crystalline base, and mentions cevadine as identical with it.

In 1891, E. Merck announced the isolation from sabadilla seeds of two new alkaloids, *sabadine*, $C_{29}H_{51}NO_8$, and *sabadinine*, $C_{27}H_{45}NO_8$.

Bearing in mind the foregoing facts, and the circumstance that the different pharmacopœias apply the name "veratrine" to a mixture of the alkaloids of sabadilla, it is desirable to discard this term altogether in its application to a definite chemical individual, and to distinguish the various alkaloids of sabadilla as follows:—

CEVADINE (Merck's "veratrine"), $C_{32}H_{49}NO_9$, crystallisable; on saponification yields cevadic acid, $C_5H_8O_2$, and cevine, $C_{27}H_{43}NO_8$.

CEVADILLINE, $C_{34}H_{53}NO_8$, amorphous; on saponification apparently yields cevadic acid, $C_5H_8O_2$, and probably cevine, $C_{27}H_{43}NO_8$.

VERATRIDINE (Wright's "veratrine"), $C_{37}H_{53}NO_{11}$, not crystallisable; on saponification yields veratric acid, $C_9H_{10}O_4$, and verine (possibly identical with cevine).

SABADINE, $C_{29}H_{51}NO_8$, crystallisable.

SABADININE, $C_{27}H_{45}NO_8$, crystallisable.

The sabadilla alkaloids give the following colour-reactions with strong sulphuric acid:—

Cevadine.—Yellow, changing to brownish-yellow and blood-red, with greenish fluorescence. On slight addition of water or prolonged exposure to air the colour changes to purple.

Veratridine.—Reacts exactly like cevadine, except that the red solution is not fluorescent.

Sabadine.—Yellowish, with green fluorescence, changing to blood-red and violet.

Sabadilline.—Permanent blood-red colour.

COMMERCIAL "VERATRINE."—*Veratrine*, B.P. is "an alkaloid or mixture of alkaloids obtained from cevadilla; not quite pure." It is described in the British Pharmacopœia of 1885 as answering to the following characters and tests:—"Pale grey, amorphous, without smell, but, even in the most minute quantity, powerfully irritating the nostrils; strongly and persistently bitter, and highly acrid; insoluble in water, soluble in spirit, in ether, and in diluted acids, leaving traces of an insoluble brown resinoid matter. It dissolves in nitric acid, yielding a yellow solution, and in sulphuric acid forming a deep red solution which exhibits a green fluorescence by reflected light. Warmed with hydrochloric acid it dissolves with production of a blood-red colour. Heated with access of air it melts into a yellow liquid, and at length burns away, leaving no residue. It is an active poison."

The "veratrine" of the United States Pharmacopœia is described as "a mixture of alkaloids" forming a white or greyish-white amorphous or semi-crystalline powder. In addition to the solubilities given in the B.P., it is said to be soluble in 2 parts of chloroform, in 6 parts of ether, and in 3 parts of cold alcohol. With nitric acid it is stated to form a yellow solution; with sulphuric acid it first yields a yellow or orange-red solution with a greenish fluorescence, which becomes more intense on addition of more acid, while the liquid is deep red by transmitted light. Heated with hydrochloric acid it dissolves with deep red colour. The German Pharmacopœia describes "veratrine" as a white powder, and states that if its solution be spread in a thin layer and powdered sugar sprinkled on it, the colour is first yellow, then green, afterwards blue, and at the end of an hour begins to disappear.[1] An examination of several samples of commercial "veratrine," which were entirely soluble in ether and otherwise answered the requirements of the German Pharmacopœia, were proved by E. Bosetti (*Archiv.* xxi. 81), to consist of a very intimate, apparently amorphous, mixture of two alkaloids, one of which was crystallisable and insoluble in water ("crystalline veratrine," Wright's "cevadine"), whilst the other was not crystallisable, but was soluble in water ("veratridine," the "soluble veratrine" of Weigelin, and of Schmidt and Köppen; Wright's "veratrine"). Relatively small quantities of the former alkaloid sufficed to render the latter insoluble in water, while the presence of a small proportion of the latter prevented the crystallisation of the former.

It is evident that commercial "veratrine" is liable to be of very variable quality and physiological activity. If the bases cevadilline, sabadine, and sabadinine be ignored, as occurring in proportions too small to affect materially the character of the article, commercial "veratrine" may be regarded as consisting essentially of cevadine and veratridine, of which cevadine is the more abundant and physiologically active constituent. No quantitative *separation* of the two bases is practicable, but a method of estimating the proportions of each in a mixture of the two could be based on the principle employed by Wright and Luff. Thus the mixture should be boiled with alcoholic soda for a moderate time, half an hour being probably ample. The liquid should then be acidulated with dilute sulphuric acid, distilled, and the distillate titrated with dilute caustic soda or baryta, and phenolphthalein. The alkali neutralised by the volatile cevadic or tiglic acid is a measure of the cevadine (and cevadilline) in the sample; while if the veratric or dimethyl-protocatechuic acid be extracted by agitating the contents of the distilling flask with ether, an estimation of the veratridine may be obtained. One c.c. of decinormal alkali neutralised by the volatile acid represents 0·0591 gramme of cevadine (and cevadilline) present; and 1 c.c. of similar alkali required by the acid subsequently extracted by ether corresponds to 0·0667 gramme of veratridine.

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[1] This test is due to Weppen. A modification of it has been described by E. Lawes (*Pharm. Zeit.*, xxxvii. 338; *Jour. Soc. Chem. Ind.*, xi. 848) consisting in the substitution of furfurol for sugar. Three or four drops of a 1 per cent. solution of furfurol in water are mixed with 1 c.c. of strong sulphuric acid, and brought in contact with the supposed veratrine. If veratrine be present, blue or dark streaks will appear in the liquid, which when mixed thoroughly assumes a dark green colour, becoming violet on warming.

CEVADINE, $C_{32}H_{49}NO_9$, is the most abundant alkaloid of cevadilla or sabadilla seeds, and, according to Wright and Luff, is also present in the rhizome of *Veratrum viride*. It is identical with the "veratrine" of Merck, and of Schmidt and Köppen, but that name is more appropriately given to the alkaloid first designated thus by Couerbe,[1] which yields veratric acid on saponification, whereas cevadine yields cevadic acid when similarly treated. But the nomenclature is liable to cause great confusion, even recent observers (*e.g.*, Ahrens, Bosetti, Merck) retaining the name "veratrine" for the base which yields cevadic acid, while the pharmacopœias apply the term "veratrine" to the mixture of alkaloids obtained from cevadilla seeds (compare page 56).

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[1] For the extraction of the alkaloids from sabadilla, Alder Wright and Luff (*Jour. Chem. Soc.*, xxxiii. 341) percolated the coarsely pulverised seeds with alcohol acidulated with tartaric acid (1 part of acid to 100 parts of seeds), evaporated the liquid to a small bulk, precipitated the resin by adding water, rendered the filtrate alkaline by sodium carbonate, and agitated with ether. The separated ether was then shaken with tartaric acid solution, and employed again. The acid liquid containing the alkaloids as tartrates was again treated with sodium carbonate, and agitated with ether, which completely dissolved the alkaloids. The ethereal solution was cautiously treated with benzoline previously diluted with a little ether until a permanent precipitate began to form, and then set aside, when the ether evaporating the more rapidly, the liquid became gradually richer in benzoline, and deposited first viscid masses of amorphous alkaloid and subsequently distinct crystals. These were stirred up with a few drops of alcohol, well drained, and slightly washed with alcohol on the filter-pump, and the nearly pure crystals of cevadine thus obtained purified by repeated recrystallisation from hot alcohol till they melted at 205°. On treating the viscid amorphous alkaloid with a quantity of ether insufficient for its complete solution, cevadilline remained behind, while on again treating the solution with benzoline and allowing it to evaporate more cevadine crystallised out. The resinoid precipitate which first separated was dissolved in dilute sulphuric acid, the liquid treated with ammonia, the precipitate drained on the filter-pump, and partially dried by exposure to air. On stirring up the nearly dry base with dilute nitric acid in a mortar, a sticky mass was obtained, which was only partially soluble in water even on boiling. The insoluble portion gradually became granular, and was filtered off and purified by boiling two or three times with small quantities of water. On treating this product with sodium carbonate and

Cevadine crystallises from alcohol in anhydrous needles, but from ether it separates only as a varnish, which becomes crystalline on moistening with slightly diluted alcohol and well stirring. The crystals are at first transparent, but on exposure to air become white and opaque, without material loss of weight. The alkaloid melts at 205° to 206°, or at a somewhat lower temperature if impure.

Cevadine dissolves in acetic ether, acetone, chloroform, amylic alcohol, and carbon disulphide, but is only sparingly soluble in petroleum spirit, even when hot. The solutions are optically inactive.

Cevadine is extremely poisonous, and exerts a peculiarly powerful action on the mucous membrane of the nose, the smallest particle producing violent sneezing.

Few of the salts of cevadine have been obtained crystallised. On adding auric chloride to a solution of cevadine in hydrochloric acid, the *aurichloride* is thrown down as a very sparingly soluble yellow precipitate, which is amorphous at first, but soon becomes crystalline. When drained and boiled with slightly diluted alcohol it dissolves, and on cooling is deposited in small, well-defined needles containing $B,HAuCl_4+2$ aqua. The water of crystallisation is lost only slowly at 100°, and the salt melts at 182°. *Cevadine picrate*, $B,C_6H_3(NO_2)_3O$, forms stable crystals, which are very slightly soluble in water, but readily in alcohol, and blacken at 225°. The *mercurichloride*, $B,HHgCl_3$, crystallises in small silvery plates, which melt at 172° with decomposition, are readily soluble in alcohol, but very slightly soluble in water. The *chloroplatinate* is an amorphous precipitate, soluble in alcohol, but decomposed by water. Alder Wright and Luff found that when cevadine was heated to 100° with twice its weight of ben-

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ether, evaporating the ethereal liquid, and treating the alkaloidal residue with dilute sulphuric acid, fine crystals of "veratrine" sulphate resembling paper pulp formed on standing. These were collected and drained on the filter-pump. On spontaneous drying by exposure to air these became a resinoid mass of conchoidal fracture, but on dissolving this in water and allowing the solution to stand, crystals were again formed from which pure "veratrine" (veratridine) was obtained by treating the solution with sodium carbonate and extracting with ether.

zoic anhydride it was converted into mono-benzoyl-cevadine, $C_{32}H_{48}(C_7H_5O)NO_9$. From the formation of this body, the impossibility of obtaining a more highly benzoylated derivative, and a study of the products of the saponification of cevadine by caustic alkali, Wright and Luff deduced the following constitutional formula for the alkaloid :—

$$(C_{27}H_{41}NO_6)\left\{\begin{array}{l}OH\\ OCO\ C(CH_3){:}CH.CH_3.\end{array}\right.$$

When cevadine is boiled with concentrated hydrochloric acid it yields tiglic acid, $C_5H_8O_2$, and a lustrous ruby-red crystalline mass, which is probably the hydrochloride of a new base. On treatment with nitric acid, cevadine is wholly oxidised; with alkaline potassium permanganate it yields acetic and oxalic acids; and with chromic acid, acetaldehyde and carbon dioxide.

Saponification of Cevadine.—When cevadine is heated in sealed tubes with water to 200° it undergoes saponification. The change occurs more readily when the alkaloid is boiled with alcoholic soda or baryta water, and is also brought about by cold aqueous caustic potash or soda, and even, though more slowly, by cold dilute ammonia. The first reaction appears to consist in the formation of angelic acid and a new base called cevine, according to the equation—

$$C_{32}H_{49}NO_9 + H_2O = C_5H_8O_2 + C_{27}H_{43}NO_8.$$

The angelic acid changes with great facility into the isomeric cevadic or tiglic acid, which is to some extent split up into acetic acid, $C_2H_4O_2$, and propionic acid, $C_3H_6O_2$, while the cevine undergoes further decomposition with the formation of non-basic resinous products. The facility with which cevadine undergoes hydrolysis is the cause of the formation of much amorphous alkaloid and other products in the extraction of the alkaloids from cevadilla seeds.

To obtain the two chief products of the saponification of cevadine, Wright and Luff boiled the alkaloid with alcoholic soda under a reflux condenser.[1] The liquid was then diluted with water, acidulated with dilute sulphuric acid, and distilled as long as any acid passed over. The distillate was neutralised with soda, evaporated to a small bulk, treated with sulphuric or phosphoric acid, and distilled. The distillate consisted partly of fluid acids, readily soluble in water, and partly of crystals or an oil becoming crystalline on standing. An alternative method is to acidulate the solution of the sodium salt with sulphuric acid, and agitate with ether. On distilling the separated ethereal layer after the ether had passed over, an acid liquid began to distil a little above 100°, the temperature quickly rising to 185°–190°, when a fraction was obtained which solidified on cooling to a mass of crystals wetted by an acid liquid. On pressing this product between blotting-paper, pearly scales of cevadic acid were obtained, melting at 64°–65°.

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[1] They continued the treatment for many hours, but this is manifestly undesirable, and half-an-hour's boiling with normal alcoholic soda is amply sufficient to effect complete saponification.

Tiglic acid, *Cevadic acid*, or *Methyl-crotonic acid*, $C_5H_8O_2$.—This acid forms triclinic prisms or scales, which melt at 64·5°, though a mixture of it with a somewhat greater weight of its isomeride, angelic acid,[1] is liquid at the ordinary temperature. Tiglic acid has an aromatic odour somewhat resembling butyric acid, but more pleasant, and boils at 198·5°, giving off a vapour which excites violent coughing. When fused with caustic potash, it yields propionic and acetic acids. *Calcium tiglate*, $Ca(C_5H_7O_2)_2 + 3$ aqua, is soluble in about 16 parts of cold water, but is much more soluble in hot water, and is deposited on cooling in white plates.

Cevine, $C_{27}H_{43}NO_8$.—In order to isolate the complementary alkaloidal product of the saponification of cevadine, Wright and Luff filtered the acid liquid left after distilling off the volatile acid, to separate resinous matter, rendered it alkaline with caustic soda, and agitated with amylic alcohol. The amylic layer, when separated, filtered, and evaporated, yielded a brownish varnish, which, on solution in dilute acetic acid, left resinous flakes. The filtrate from these, on fractional treatment with soda and amylic alcohol, gave an amber-coloured varnish of cevine, perfectly soluble in acids. When heated in a capillary tube, this did not frit below 140°, and fused at 145°. It dissolved readily in alcohol and amylic alcohol, sparingly in chloroform, and hardly perceptibly in ether. Neither free cevine nor its salts were obtained crystallised. On adding Mayer's reagent to a solution of the base in acetic acid, nearly insoluble white flakes were precipitated, containing (after drying at 100°) $B,HHgI_3$. The aqueous solution of cevine becomes turbid on warming. Cevine does not attack the mucous membrane, gives a crimson colour with strong sulphuric acid, and yields a brown colour with sulphuric acid and sugar.

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[1] ANGELIC ACID, or PENTENOIC ACID, isomeric with tiglic acid, crystallises in long prisms, having an aromatic smell, melting at 44°–45°, and boiling at 185°. When boiled for some time, or when heated to 100° with sulphuric acid, it is converted into *tiglic acid*. Angelic acid is but slightly soluble in cold water, but dissolves readily in hot water and alcohol, and is extracted from aqueous liquids by agitation with ether. When fused with caustic potash, angelic acid behaves like tiglic acid. *Calcium angelate*, $Ca(C_5H_7O_2)_2 + 2$ aqua, is much more soluble in cold water than in hot. A cold saturated solution contains about 23 per cent. of the salt, but when heated to 30°–40° glistening needles separate out, and at about 70° the whole becomes semi-solid. If air has been excluded, the crystals re-dissolve completely on cooling. The constitution of angelic and tiglic acids is probably represented by the following formulæ :—

$CH_2 : C(CH_3).CH_2.COOH.$
Angelic acid

$CH_3.CH : C(CH_3).COOH.$
Tiglic acid.

VERATRIDINE, $C_{37}H_{53}NO_{11}$,[1] occurs in sabadilla seeds, and possibly in minute quantity in the rhizomes of white and green hellebore.[2] It is identical with Wright and Luff's "veratrine."

Veratridine free from cevadine has never been obtained crystallised. It melts in a capillary tube at 180°.

On triturating solid veratridine with dilute nitric acid a horny *nitrate* is formed, which is almost insoluble in water, even when boiling. Dilute sulphuric acid readily dissolves veratrine, and, on standing, the *sulphate* crystallises out in extremely fine needles, which, on drying, unite to form a horny translucent mass, reproducing crystals when dissolved in water and allowed to stand. The *hydrochloride* exhibits a similar behaviour, but the crystals are not so well marked and distinct. The *aurichloride* is obtained as a gelatinous yellow precipitate, which, when dried over sulphuric acid, becomes a translucent horny mass, not crystallisable from alcohol.

When boiled with alcoholic soda, veratridine undergoes saponification, with formation of veratric or dimethyl-procatechuic acid and verine, according to the equation:—

$$C_{37}H_{53}NO_{11} + H_2O \quad = \quad C_9H_{10}O_4 + C_{28}H_{45}NO_8.$$

The *acid* is identical with that obtained by the saponification of pseudaconitine (Part II. p. 218). The *basic product* verine, or veratroïne, presents a close resemblance to cevine, obtained in a similar manner from cevadine. The only recognisable distinctions observed by Wright and Luff were in the behaviour on heating and the elementary composition. Thus, verine fritted in the water-bath or in a capillary tube at 95°, and on raising the temperature, gradually became a thick viscid mass, exhibiting no definite melting-point, and not becoming completely fluid till heated to about 130°; whilst cevine exhibited no sign of fritting below 140°, and completely fused at 145°.[1]

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[1] The preparation of pure veratridine is described in a footnote on page 58.

[2] See footnote on page 66.

Bosetti (*Arch. Pharm.*, [3], xxi. 81) attributes to veratridine the formula $C_{32}H_{49}NO_9$, and the melting-point 150°–155°. To the basic product of its saponification he gives the formula $C_{55}H_{92}N_2O_{16}$, and states its melting-point at 143°–148°.

Sabadine, $C_{29}H_{51}NO_8$, has been recently isolated from sabadilla seeds by E. Merck (*Chem. Zeit. Rep.*, xv. 48; *Jour. Soc. Chem. Ind.*, x. 481). It is deposited by the slow evaporation of its alcoholic solution in well-defined crystals, which melt with decomposition at 238°–240° C.; but the residue obtained by evaporating the ethereal solution has no definite melting-point. The crystals are difficultly soluble in water and ether, and insoluble in petroleum-spirit. With strong sulphuric acid, sabadine gives a yellowish coloration and green fluorescence, the colour subsequently changing to blood-red, and finally to violet. Concentrated nitric acid appears to produce no change. B,HCl crystallises with 2 aqua, but becomes anhydrous with decomposition at 282°–284°. Sabadine is not precipitated on adding caustic alkalies, alkaline carbonates, or ammonia to cold solutions of its salts, but is separated in a flocculent form on warming the liquid. It can be extracted from the alkaline liquid by agitation with ether. Sabadine attacks the mucous membrane of the nose and causes sneezing, but in a less marked degree than cevadine.

Sabadinine, $C_{27}H_{45}NO_8$, behaves like sabadine with alkalies. It is best extracted by agitation with chloroform. From its ethereal solution it separates in hair-like needles, which commence to melt at 160°, and decompose at a higher temperature. The alkaloid is moderately soluble in water, sparingly in ether, but readily in alcohol. Concentrated sulphuric acid produces a permanent blood-red coloration, but nitric acid causes no visible change. BHCl forms crystals which contain water, and are readily soluble. Sabadinine does not occasion sneezing.

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[1] Wright and Luff, to whom the foregoing observations are due, point out that the numbers obtained by the analysis of veratrine are not incompatible with other formulæ differing but little from $C_{37}H_{53}NO_{11}$, which is that agreeing best with their results. But they add that the formula $C_{28}H_{51}NO_{11}$ would indicate cevine and verine as being identical, and cevadine and veratridine as containing a common radical when their formulæ are thus written :—

Veratridine, $(C_{27}H_{42}NO_7)O.CO.C_6H_3(O.CH_3)_2$.
Cevadine, $(C_{27}H_{42}NO_7)O.CO.C(CH_3):C_2H_4$.

Alkaloids of the Hellebores.[1]

The rhizome of white hellebore, *Veratrum album*, was found by Pelletier and Caventou, in 1820, to contain an alkaloid which they assumed to be identical with that obtained by them from cevadilla seeds, and named *veratrine*. In 1837, E. Simon confirmed the presence of veratrine in white hellebore, and discovered a second alkaloid, *jervine*, readily crystallisable, and remarkable for the insolubility of its sulphate. In 1842, Weigand confirmed the presence of jervine and veratrine in white hellebore. Weppen arrived at the same conclusion in 1872. In 1872, Peugnet confirmed Simon's discovery of jervine, but disputed the identity of the second alkaloid with the veratrine of sabadilla seeds, believing it to be identical with the base *veratroïdine*, which had been found by Bullock in green hellebore. Mitchell, in 1874, claimed that this amorphous base was neither veratrine nor veratroïdine, and called it *veratralbine*; but, in 1876, Wormley obtained from both white and green hellebore a base which agreed in all its reactions with the so-called veratrine (cevadine) of sabadilla. In 1877, white hellebore was again investigated by Tobien, who found *jervine*, to which he ascribed the formula $C_{27}H_{47}N_2O_8$, and an amorphous base, *veratroïdine*, $C_{24}H_{37}NO_7$. In 1879 (*Jour. Chem. Soc.*, xxxv. 405), Alder Wright and Luff, as the result of an able investigation, announced the root of white hellebore to contain the crystallisable alkaloids *jervine*, $C_{26}H_{37}NO_3$; *pseudojervine*, $C_{29}H_{43}NO_7$; *rubijervine*, $C_{26}H_{43}NO_2$; an amorphous alkaloid "*veratralbine*" having a composition approximating to the formula $C_{26}H_{43}NO_5$; and a minute quantity of "*veratrine*" (veratridine) $C_{37}H_{53}NO_{11}$, to the presence of which last they attributed the sternutatory properties of the root. In 1890, C. Pehkschen (*Pharm. Zeit. Russ.*, xxix. 339; *Jour. Chem. Soc.*, lx. 87) isolated from the rhizome of white hellebore the alkaloids *veratroïdine*, $C_{32}H_{53}NO_9$; *jervine*, to which he attributed the formula $C_{14}H_{22}NO_2$; and *pseudojervine*, $C_{29}H_{49}NO_{12}$. The alkaloids of *Veratrum album* have recently been reinvestigated (1885 to 1890) by G. Salzberger (*Arch. Pharm.*, ccxxviii. 462; abst. *Pharm. Jour.*, [3], xxi. 745, 899), on very large amounts of material. He confirms Wright and Luff's descriptions of jervine, pseudojervine, and rubijervine, but doubts the existence of veratralbine as a chemical individual. He further isolated two new crystallisable alkaloids, *protoveratrine*, $C_{32}H_{51}NO_{11}$, and *protoveratridine*, $C_{26}H_{45}NO_8$, besides a small quantity of an unnamed crystallisable *base* containing $C_{26}H_{45}NO_{10}$. Salzberger points but that jervine has only a slight toxic action, and pseudojervine is absolutely inactive. He attributes the sternutatory property of *Veratrum album* to protoveratrine, which is intensely poisonous, and excites most violent sneezing; while protoveratridine, on the other hand, is very bitter, but not poisonous, and is probably a decomposition-product of protoveratrine.

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[1] See footnote on page 54.

Green or American Hellebore, *Veratrum viride*, familiarly known as "Indian Poke," contains in the main the same alkaloids as *Veratrum album*. In 1838, H. Worthington announced it to contain "an alkaloid substance identical with *veratrine*." In 1857, J. G. Richardson concluded that "not only in its physical characters, but also in its chemical actions, the alkaloid of *Veratrum viride* is identical with veratrine of *V. sabadilla*." In 1862, J. G. Scattergood announced the presence of a second alkaloid, insoluble in ether, and a resinous substance to which the sedative action of the drug was mainly attributable. In 1864, S. R. Percy extracted from green hellebore an alkaloid which he concluded had all the properties of veratrine from sabadilla seeds. On the other hand, in 1865, C. Bullock claimed that the alkaloid of green hellebore was not identical with veratrine, as it did not respond to the colour-reactions for that alkaloid; that the resin of Scattergood owed its activity to the presence of another alkaloid; and that these two principles exhibited the same reactions with mineral acids and with other reagents, but differed in their fusing-points and in their behaviour to ether, in which one was soluble and the other insoluble. For these two alkaloids of green hellebore, G. B. Wood proposed the names *veratroïdine* and *viridine*. In 1872, Peugnet also concluded that the former of these bases was distinct from veratrine, as it did not respond to the sulphuric acid test for that substance, though it did to Trapp's test with hydrochloric acid, and he pointed out Bullock's viridine was identical with Simon's *jervine* from *Veratrum album*. This was independently proved by C. L. Mitchell in 1874, who prepared jervine from green hellebore, fully described its properties and reactions, and stated that it was not contained in cevadilla seeds. He also obtained a base soluble in ether, which he concluded was distinct from veratrine, as it did not behave like that base with the mineral acids. In 1876, T. G. Wormley also prepared jervine from the roots of green and white hellebore, and concluded that both roots contained an alkaloid which, when pure, fully responded to all the reactions of *veratrine*. In the same year, C. Bullock concluded that *jervine* was the only alkaloid in the root of green hellebore, and that the so-called veratroïdine (or, according to Wormley, veratrine) was a mixture of jervine with a light coloured resin, the presence of which rendered the alkaloid more or less soluble in ether. The reaction of the alkaloid with sulphuric acid, regarded by Wormley and others as due to veratrine, he attributed to a resin soluble in ether, which adhered to the alkaloid with great persistency. Bullock also examined some of the products obtained by Scattergood in 1862, and considered the preparations labelled "veratrine" to consist of jervine mixed with the peculiar resin which produced a mahogany-red coloration with sulphuric acid, and he regarded veratroïdine as of similar composition. These very contradictory observations were reviewed by Alder Wright and Luff, who, in 1879 (*Jour. Chem. Soc.*, xxxv. 421), reinvestigated the subject, and, by means of improved methods, found the rhizome of *Veratrum viride* to contain the same five alkaloids they had isolated from white hellebore, and, in addition, a base they called *cevadine*, identical with Merck's veratrine from cevadilla seeds.

The proportions of the different alkaloids isolated by Wright and Luff from 1000 parts of the roots of white and green hellebore were as follow :—

Alkaloid.	Formula.	*Veratrum album.*	*Veratrum viride.*
Jervine, . . .	$C_{26}H_{37}NO_3$	1·3	0·20
Pseudojervine, .	$C_{29}H_{43}NO_7$	0·4	0·15
Rubijervine, . .	$C_{26}H_{43}NO_2$	0·25	0·02
Veratralbine, . .	$C_{28}H_{43}NO_5$	2·2	traces
Veratridine[1] . . ("Veratrine"),	$C_{37}H_{53}NO_{11}$	0·05	less than ·004
Cevadine, . . .	$C_{32}H_{49}NO_9$	apparently absent	0·43
		4·20	0·80

The proportion of total alkaloids obtained by Wright and Luff from green hellebore (=0·08 per cent.) is very much lower than that isolated by other observers. Thus C. L. Mitchell found in three specimens 0·49, 0·61, and 0·69 per cent., of which 0·23, 0·26, and 0·29 consisted of jervine. Bullock obtained 0·66 per cent., while Farr and R. Wright have recently recorded from 0·16 to 1·20 per cent., with an average of 0·73, of which 0·22 was jervine.

The following table shows the formulæ and leading properties of the characteristic alkaloids of the hellebores, according to the descriptions of Alder Wright and Luff and of G. Salzberger, and, for veratralbine, of C. Pehkschen :—

[1] See footnote on page 68.

	Jervine.	Rubijervine.	Pseudojervine.	Protoveratrine.	Protoveratridine.	Veratralbine (Veratroïdine).
	$C_{26}H_{37}NO_3$	$C_{26}H_{43}NO_2$.	$C_{29}H_{43}NO_7$.	$C_{32}H_{51}NO_{11}$.	$C_{26}H_{45}NO_8$.	$C_{28}H_{43}NO_5$
e form.	Satiny prismatic crystals.	Long prisms resembling jervine.	Thin hexagonal tables or prisms resembling jervine.	Rectangular or hexagonal plates, or shining prisms.	Quadrilateral plates.	Amorphous.
ical action.	Moderately poisonous.	Inactive.	Inactive.	Very poisonous; violent sneezing.	Bitter; not poisonous.	...
oint ; ° C.	237-242.	240-246.	299-307.	245-250.	265.	149.
:—						
.	Soluble.	Sparingly.	Sparingly.	Sparingly.	Very sparingly.	Very soluble.
	Sparingly sol.	Very sparingly.	Insoluble.	Very sparingly.	Insol.	Readily sol.
orm.	Soluble.	Soluble.	Soluble.	Sparingly.	Very sparingly.	Readily sol.
um spirit.	Almost insol.	Very slightly.	Very slightly.	Insol.	...	...
e.	Almost insol.	Soluble.	Sparingly.	Insol.	Insol.	Readily sol.
to litmus,	Alkaline.	Alkaline.	Alkaline.	Alkaline.	...	...
e.	Crystalline; nearly insol.	...	Crystalline. Readily sol.	...	...	Amorphous.
	Crystalline; nearly insol.	...	...	...	...	Amorphous.
hloride.	Crystalline; very sparingly sol.	...	Very sparingly sol.	...	...	Amorphous.
nts :—						
hloride.	Ppte.; crystalline.	Ppte.	Flocculent ppte.	Amorphous golden-yellow ppte.	...	...
chloride,	Granular ppte. Pale orange-red.	No reaction.	No reaction.	No reaction.	No reaction.	...
is.	Fine needles insol. in excess.	Gelatinous ppte.	Cheesy ppte.	On warming, cryst. ppte.	Cryst. ppte.	...
o-cadmium e.	Ppte.	Ppte.	Ppte.	Ppte.	No reaction.	...
solution.	Ppte.	Ppte.	Ppte.	Ppte.	Ppte.	Ppte. if not too dilute.
otungstic	Ppte.	Ppte.	Ppte.	Ppte.	Ppte.	...
cid.	Ppte.	Ppte.	Ppte.	Ppte.	Ppte.	...
	Turbidity.	No reaction.	Ppte.	No reaction.	Ppte.	...
actions :—						
sulphuric	Yellow, brownish-yellow, bright green.	Yellow; then orange and dark red.	Yellow; then bright green.	Greenish, blue, and finally violet.	Violet, changing to cherry-red.	Yellow, then orange-red and blood-red, with green fluorescence.
ic acid and	Violet, then blue.	...	...	Green, olive-green, dark brown.	...	Brown.
hloric acid arming).	No coloration.	...	...	Cherry-red and odour of isobutyric acid.	Bright red and odour of isobutyric acid.	Rose colour
cid.	...	...	...	...	...	Rose, quickly changing to pale yellow.

No examination of the rhizome of *Veratrum viride* on the lines of Salzberger's investigation of *V. album* appears to have been made.[1] Dragendorff has found *jervine* in *Veratrum nigrum*, and Tobein (1877) obtained *jervine* and "*veratroïdine*" from the young leaves of *Veratrum lobelianum*.[2]

For the extraction of the total alkaloids from the rhizome of *Veratrum viride*, Farr and Wright (*Chemist & Druggist*, Oct. 29th, 1892) recommend the exhaustion of the drug with alcohol of 60–70 per cent. Of the tincture obtained, which, if made of B.P. strength, with 4 oz. of root to the pint of spirit, contains on the average 0·143 gramme of total alkaloid per 100 c.c., 50 c.c. is evaporated in porcelain at 100°, with addition of water, till the alcohol is driven off. The residual liquid is slightly acidulated with hydrochloric acid, and filtered through cotton-wool from the precipitated resin into a glass separator. The separated resinous matter is found to retain alkaloid, and is therefore redissolved in a little rectified spirit, the solution diluted with acidulated water, the alcohol evaporated, and the liquid filtered into the separator. The mixed filtrates are rendered distinctly alkaline with ammonia, and the alkaloid extracted by agitation with chloroform, using first 10 c.c., and then two successive quantities of 5 c.c. The chloroform is separated and agitated with successive small quantities of 1 per cent. hydrochloric acid. The acid liquid separated from the chloroform is again made alkaline with ammonia, and the alkaloid shaken out with 15 c.c. of chloroform used in three portions. On evaporating the separated chloroform and drying the residue at 100°, the veratrum alkaloids are obtained in a semicrystalline condition, usually entirely soluble in 2 per cent. acetic acid, but occasionally yielding a slightly turbid and coloured solution, owing to the presence of resinous matter.[1]

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[1] *Veratrum viride* and *V. album* are commonly called green and white hellebore. They belong to the *Melanthaceæ*, and contain jervine and other well-defined basic principles. On the other hand, *Helleborus fœtidus* and *H. niger*, the black hellebore or Christmas rose, belong to the *Ranunculaceæ*, and contain the poisonous glucosides *helleborin* and *helleboreïn*.

HELLEBORIN, $C_{36}H_{42}O_6$, forms white glittering needles, which, if placed on the tongue, are almost tasteless, but if dissolved in alcohol and then tasted produce a burning numbing sensation. Strong sulphuric acid dissolves helleborin with intense red coloration, which gradually disappears, a white powder separating. By hydrolysis, helleborin is split into glucose and helleboresin. Helleborin may be extracted from hellebore root by alcohol, and exhibits toxic properties similar to those of digitalis.

HELLEBOREÏN, $C_{26}H_{44}O_{15}$, forms fine hygroscopic needles, which are bitter and excite sneezing. They are soluble in water and dilute alcohol, but not in ether. Strong sulphuric acid colours helleboreïn golden-yellow, changing to reddish-brown. On hydrolysis, helleboreïn splits into glucose and helleboretin, a body which when moist is violet-blue, but on drying becomes dirty green. Helleboreïn is present in the seeds and leaves of hellebore, but not in the root.

[2] Robbins (*Proc. Amer. Pharm. Assoc.*, 1877, pp. 439, 523) isolated from green hellebore a crystallised alkaloid which he called veratridine. It possessed a physiological action similar to that of veratrine (cevadine ?) though in a less degree. Its solution in concentrated sulphuric acid is at first yellow, changing quickly to a pink-red, and after standing for some hours assumed a clear indigo-blue colour, very similar to that described by Weppen as yielded by veratrine (cevadine ?) if mixed with sugar.

To estimate the *jervine* and distinguish it from the accompanying alkaloids obtained in the foregoing process, Farr and Wright proceed as follows:—The alkaloidal residue is dissolved in 2 per cent. acetic acid, and filtered, if turbid. A measured quantity of the solution is then treated with a few grains of potassium nitrate, shaken, and allowed to stand for some time. The clear liquid is then removed with a pipette, the crystals washed with a little water, and the latter drawn off when clear. The mixed liquids are measured, made alkaline with ammonia, and agitated with chloroform, which is separated, evaporated, and the residual alkaloid dried and weighed. From the weight obtained, a deduction is made of 0·005 gramme for every 6 c.c. of liquid, as a correction for the solubility of the jervine nitrate, and this correction is added to the weight of jervine obtained by treating the precipitated nitrate with ammoniated water and chloroform, and separating and evaporating the latter.

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[1] "The process presents no great difficulty in working, but great care is needed in order to secure perfect extraction of the alkaloids from the chloroformic solution by means of acidulated water. This appears to arise from the tenacity with which the resinous matter present adheres to the alkaloid, but may also be due in part to the sparing solubility of the alkaloidal salts produced. In some instances it was found necessary to employ fourteen or fifteen successive portions of acidulated water for the shaking-out process before it came away free from alkaloid; it is therefore very important that the process should be repeated until the final washings give no reaction with Mayer's reagent. The process may be considerably shortened by taking the first chloroformic alkaloidal solution, adding 1 c.c. normal HCl and 10 c.c. water, evaporating over a water-bath with constant stirring until all chloroform has been removed; filtering from particles of resinous matter, washing the latter with acidulated water until the washings come away free from alkaloid; mixing the liquids, making the solution alkaline with ammonia, shaking out the alkaloids with 15 c.c. chloroform added in three portions, drawing off the latter into a dish, and evaporating, drying, and weighing. This modification gives slightly higher results, but the final residue is more highly coloured than that obtained by the original process." —Farr and Wright.

Jervine, $C_{26}H_{37}NO_3$,[1] is the principal crystalline alkaloid of *Veratrum album*, in which Alder Wright and Luff found 1·3 per cent. It is also present in the rhizome and other parts of American or green hellebore, *Veratrum viride*, and according to Tobien, exists in *V. lobelianum* (see footnote, page 68).

The method of isolating jervine from white hellebore root has already been described.

Jervine crystallises from its solution in boiling alcohol in beautiful satiny prismatic needles, generally arranged in tufts, bundles, and stellar groups, having a characteristic microscopic appearance. The solutions of jervine are slightly lævo-rotatory.

Jervine melts at 237°–239°, according to Wright and Luff; at 237·7°, according to Pehkschen; and at 238°–242°, according to Salzberger. It is almost insoluble in water, acetic ether, benzene (1 : 1658), and carbon disulphide, and wholly insoluble in petroleum-ether. It is fairly soluble in acetone and amylic alcohol. At 25° C. it dissolves in 17 parts of absolute alcohol, and in 60 of chloroform. On spontaneous evaporation of its chloroformic solution, jervine is usually obtained as a transparent vitreous mass, which immediately crystallises if touched with a drop of alcohol. Crystallised jervine requires 268 parts of ether for solution, but its solubility is greatly increased by the presence of amorphous alkaloids (*e.g.*, veratralbine), and it is readily extracted by ether from alkaline aqueous liquids.

Jervine is not affected by prolonged boiling with alcoholic potash. It does not produce sneezing, and is only moderately toxic.

Jervine is a well-defined base, having an alkaline reaction to litmus. It forms readily crystallisable *salts* with acids. The acetate and phosphate are readily soluble in water, but the hydrochloride, nitrate, and sulphate dissolve very sparingly, and are still less soluble in presence of the corresponding free acids, and hence may be precipitated thereby from their aqueous solutions. They may also be obtained by precipitating a solution of jervine acetate or phosphate with chloride, sulphate, or nitrate of alkali-metal. The precipitates rapidly assume a crystalline structure.

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[1] The formula of jervine is differently given as follows, and it is not certain that the so-called jervine has always been the same substance:—

From *Veratrum album*, $C_{30}H_{46}N_2O_3$ (probably contained pseudojervine). Will, *Ann. Chem. Pharm.*, xxxv. 116.

From *V. lobelianum*, $C_{27}H_{47}N_2O_3$. Tobien (1877), *Pharm. Jour.*, [3], viii. 808.

From *V. album* and *V. viride*, $C_{26}H_{37}NO_3+2H_2O$. Alder Wright and Luff (1879), *Jour. Chem. Soc.*, xxxv. 405.

From *V. album*, $C_{14}H_{22}NO_2$. Pehkschen (1891), *Jour. Chem. Soc.*, lx. 87.

The formula of Wright and Luff has been fully confirmed by G. Salzberger (*Arch. Pharm.*, ccxxviii. 462; abst. *Pharm. Jour.*, [3], xxi. 901), and no doubt represents the true composition of jervine.

Jervine sulphate, B_2,H_2SO_4, is readily obtained by treating a solution of jervine acetate with excess of dilute sulphuric acid, or by treating free jervine with the dilute acid (1 : 5). In the first case it is obtained as an immediate precipitate, which rapidly becomes crystalline. In the latter case, the alkaloid does not dissolve, but is converted into an indistinctly crystalline, gelatinous magma, almost insoluble, even after washing, either in cold or boiling water. This property was utilised by Wright and Luff to separate admixed rubijervine, which resembles jervine in being, in presence of amorphous bases, somewhat soluble in ether, while pseudojervine is much less soluble under such circumstances.[1]

When treated with concentrated sulphuric acid, solid jervine dissolves with yellow coloration, which changes in succession to dark yellow, brownish-yellow, and greenish-brown. After standing some time, the mixture assumes a bright green colour, which ultimately disappears, and dirty white or brownish flakes separate, which may become granular or crystalline. The green shades of colour are due to absorption of moisture, and hence, when the test is made in porcelain, the colour is first observable at the edges, and finally extends to the whole liquid, which becomes a dark green. When a test-tube is employed, the green colour is not developed for some hours, but may be immediately produced from the liquid at its greenish-brown stage by slightly diluting with water, the tint becoming successively olive-green, dark chrome-green, and dark emerald, as more water is added. With a further quantity of water the tint becomes lighter, until finally a nearly colourless liquid results, with a few brownish flakes suspended therein. This colour-reaction is peculiar to jervine and pseudojervine. The other alkaloids of hellebore dissolve in cold concentrated sulphuric acid with yellow colour, rapidly becoming brown-yellow, brown, reddish-brown, and finally more or less red in tint; in this respect presenting some resemblance to the behaviour of veratridine and cevadine (page 56).

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[1] RUBIJERVINE, $C_{26}H_{43}NO_2$, is deposited from alcoholic solution in anhydrous crystals, which melt when pure at 236°. It is readily dissolved by dilute hydrochloric acid, but on adding strong hydrochloric acid to the solution, the *hydrochloride* separates as a crystalline magma readily soluble in water. The *sulphate* is readily soluble in hot water, or in cold water containing free sulphuric acid, but less readily in cold water free from acid. Rubijervine dissolves in concentrated sulphuric acid to a clear yellow liquid, becoming successively dark yellow, brownish-yellow, and brownish blood-red, changing after several hours to a brownish-purple. On slightly diluting the brownish blood-red liquid with water, it becomes successively crimson, purple, dark lavender, dark violet, and ultimately light indigo. Other characters and reactions of rubijervine are given on page 67.

PSEUDOJERVINE, $C_{29}H_{43}NO_7$, forms crystals much resembling jervine, but anhydrous, and melting at a considerably higher temperature (299° C.). The *sulphate* is only sparingly soluble in cold water, but readily in hot water, while the *hydrochloride*, B,HCl, 2 aqua, is only sparingly soluble in ether, or cold or hot water, but is much more readily dissolved by water slightly acidulated with hydrochloric acid. Exactly the reverse is the case with jervine and rubijervine hydrochlorides. With strong sulphuric acid, pseudojervine behaves in exactly the same manner as jervine.

Sulphuric acid reacts with the sulphate, hydrochloride, and acetate of jervine in the same manner as with the free alkaloid, but it dissolves the nitrate with orange-red colour, which is permanent for several hours.

With Fröhde's reagent, jervine gives a green coloration similar to that produced by sulphuric acid alone.

With sulphuric acid and sugar (compare Part ii. page 315), jervine gives a violet coloration, changing to blue; a reaction said by Pehkschen to distinguish it from veratroïdine (veratralbine), which gives a brown coloration when similarly treated.

Nitric acid dissolves jervine with pinkish coloration to a nearly colourless liquid, which often deposits crystals of the nitrate.

Strong hydrochloric acid gives no coloration with jervine, but immediately converts it into a more or less crystalline hydrochloride, which is insoluble in the acid.

VERATRALBINE or VERATROÏDINE is the principal amorphous alkaloid of white hellebore root. According to Alder Wright and Luff, the composition approximates to the formula $C_{28}H_{43}NO_5$, while according to C. Pehkschen it contains $C_{32}H_{53}NO_9$, who states that it melts at about 149° and chars at 172°, is optically inactive, and dissolves in alcohol in almost all proportions. It dissolves in 9 parts of ether, 6 of chloroform, or 13 of benzene. The hydrochloride, hydrobromide, sulphate, nitrate, oxalate, and acetate are amorphous. Veratroïdine gives precipitates with most of the general reagents for alkaloids. With concentrated sulphuric acid, veratroïdine gives a yellow coloration which changes to orange-red and blood-red, with a strong green fluorescence, while concentrated nitric acid produces a transient rose colour, which soon changes to citron-yellow. Hydrochloric acid (11 per cent. is the preferable strength) gives a beautiful rose coloration, which distinguishes the base from veratridine.

With sulphuric acid and sugar veratralbine gives a brown coloration.

The colour-reactions of veratralbine are closely akin to those developed by cevadine, and far more resemble those produced by rubijervine than the reactions yielded by jervine or pseudojervine. Veratralbine does not cause sneezing, and is unchanged by prolonged treatment with boiling alcoholic potash.

PROTOVERATRINE, $C_{32}H_{51}NO_{11}$, is described by Salzberger (*Arch. Pharm.*, ccxxviii. 230) as crystallising from dilute solutions in microscopic, quadrilateral plates, which melt with charring at 245°–250°. It is insoluble in water, benzene, and petroleum spirit, but sparingly soluble in chloroform and boiling alcohol. Cold ether scarcely touches it, but when boiling takes up a little more. It is soluble in dilute acids, with the exception of acetic acid. Concentrated sulphuric acid dissolves protoveratrine slowly with green coloration, which passes to cornflower-blue, and in some hours to violet. When warmed with sulphuric acid, the coloration is first light and then dark cherry-red, while an odour of isobutyric acid is evolved. Concentrated hydrochloric and phosphoric acids produce the same reaction. With sulphuric acid and sugar, protoveratrine gives an olive-green coloration, becoming dirty green, and finally dark brown. Solutions of salts of protoveratrine are precipitated by ammonia, Nessler's solution, Mayer's reagent, potassio-cadmium iodide, phosphotungstic acid, and picric acid; but not by tannin, platinic chloride, nor mercuric chloride. $B,HAuCl_4$ is a golden yellow, amorphous precipitate. Protoveratrine is exceedingly poisonous. A minute amount applied to the nose causes violent sneezing. The behaviour of protoveratrine with saponifying agents has not been recorded, but the large percentage of oxygen, and the fact that protoveratridine, $C_{26}H_{45}NO_8$, appears to be a decomposition-product, renders it highly probable that it can be hydrolysed.

It is probable that protoveratrine is the sole sternutatory alkaloid of *Veratrum album*, as veratridine was not actually isolated therefrom by Wright and Luff, but its existence inferred from the formation of veratric acid.

Protoveratrine can be readily extracted from white hellebore by cold water, but to obtain the crystalline base the rhizome should be freed from fatty and resinous matters by treatment with ether, and then exhausted with alcohol. The spirit is evaporated in a vacuum, the residue treated with water acidulated with acetic acid, rapidly filtered, and the filtrate treated with solid metaphosphoric acid (glacial phosphoric acid), as long as a precipitate is produced. The liquid is filtered from the bulky precipitate (which contains jervine, rubijervine, and much amorphous matter), made alkaline with ammonia, and shaken with ether. The ethereal extract is distilled, when protoveratrine crystallises out, and may be purified from a little jervine and rubijervine by recrystallisation from alcohol. By this process, Salzberger obtained 0·03 per cent. from white hellebore root. If the ammoniacal solution be subsequently shaken with chloroform, pseudojervine is extracted.

PROTOVERATRIDINE, $C_{26}H_{45}NO_8$, is a non-poisonous base isolated by Salzberger from white hellebore root.[1] It crystallises in colourless, four-sided plates, melting at 265°, and is almost insoluble in alcohol, methyl alcohol, acetone, or chloroform, and quite insoluble in ether, benzene, and light petroleum. It does not cause sneezing, but its solutions in acids are very bitter, and give a crystalline precipitate with ammonia. With concentrated sulphuric acid, the base yields a violet coloration, changing to cherry-red. Its solution in strong hydrochloric acid (like that of veratridine) becomes light red on warming, and evolves a decided odour of isobutyric acid. The solution of the sulphate gives copious precipitates with phosphotungstic, tannic, and picric acids, and with Mayer's reagent. B_2,H_2PtCl_6 is soluble in water, but is precipitated in large hexagonal plates on adding alcohol to mixed solutions of platinic chloride and protoveratridine hydrochloride.

Alkaloids of the Potato, &c.

SOLANINE, $C_{42}H_{75}NO_{15}$(?), occurs in *Solanum nigrum*, and has been found in the bitter-sweet (*Solanum dulcamara*) and other species of *Solanum*, including the potato[2] (*Solanum tuberosum*).

For the extraction of solanine from potato-seeds, the material should be macerated in water slightly acidulated with sulphuric acid, the liquid heated, and treated with ammonia. A precipitate of impure solanine is formed, which is dried thoroughly and exhausted with boiling alcohol. On cooling, the solanine is almost entirely deposited, and is purified by several recrystallisations. A pure product is obtainable more readily if the acid extract of the seeds be treated with lead acetate and filtered, and the solanine subsequently precipitated by ammonia or milk of lime.

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[1] The coarsely powdered rhizome was mixed with water and baryta, and the liquid extracted with ether. From the solution the ether was evaporated at the lowest possible temperature in a current of hydrogen. On standing, the resultant dark green syrup gave a crop of crystals mostly consisting of jervine.

[2] R. Firbas (*Monatsh. Chem.*, x. 541) macerates fresh potato-shoots in water containing 2 per cent. of acetic acid, renders the filtered liquid alkaline with ammonia, and extracts the precipitate (after drying) with boiling rectified spirit. The liquid is filtered hot, and treated with a little ammonia till just turbid. On cooling, crystals of solanine are deposited, and later, an amorphous base called solaneine is deposited. To this body Firbas attributes the formula $C_{52}H_{83}NO_3+4H_2O$; solanine being $C_{52}H_{93}NO_{18}+4\frac{1}{2}H_2O$.

SOLANEINE is said to melt at 208°, the melting-point of solanine being stated at 244° C. When hydrolysed by dilute hydrochloric acid, solaneine behaves like solanine, yielding solanidine, $C_{40}H_{61}NO_2$, and a glucose said to be distinct from ordinary dextrose or lævulose.

Solanine deposited from hot alcohol forms fine, silky crystals, appearing under the microscope as rectangular prisms, but when obtained by precipitation it forms gelatinous flocks, which on drying agglomerate to a horny mass.

Solanine melts at 235–240°, and at a slightly higher temperature decomposes with an odour of caramel, giving a sublimate of solanidine.

Solanine is odourless when dry, but when moist exhales an odour recalling that of potatoes while cooking. The taste of solanine is somewhat bitter and pungent. It leaves on the pharynx a persistently acrid sensation. Solanine is very poisonous, producing in dogs and cats violent vomiting, followed by somnolence, and sometimes accompanied by paralysis of the lumbar muscles. One grain killed a rabbit in six hours, and $\frac{1}{4}$ grain is strongly nauseating to a man. Solanine is stated by Sardas to be an excellent neurotic sedative, more efficacious in long-standing neuralgia, especially when neuritis is present, than either antipyrine or antifebrin.

Solanine is nearly insoluble in water, and only very slightly soluble in cold alcohol, but dissolves readily in hot alcohol. It is insoluble in ether, chloroform, benzene, or petroleum-spirit, but is soluble in amylic alcohol, which may be employed to extract solanine from its alkaline solutions.

Solanine is said to have a faintly alkaline reaction. It is a very feeble base, the salts being mostly decomposed by excess of water. The *acid sulphate*, however, is said to be very stable and not decomposed by water, even on heating, in contra-distinction to the neutral salt. It is amorphous and very bitter. The *hydrochloride* is precipitated as a jelly on adding ether to a solution of solanine in alcohol acidulated with hydrochloric acid. B_2,H_2PtCl_6 is a yellow flocculent precipitate, insoluble in ether, but readily soluble in hot water or in alcohol. The chromate, phosphate, and oxalate of solanine have been obtained crystallised.

Solanine is not affected by alkalies, even when boiling. It does not reduce Fehling's solution, but reduces ammonio-nitrate of silver on heating.

Concentrated nitric acid dissolves solanine in the cold to a liquid, which is at first colourless, but rapidly acquires a magnificent purple colour, which soon disappears. With strong hydrochloric acid, solanine gives a yellow coloration. Concentrated sulphuric acid dissolves solanine with orange colour, changing to deep violet and brown.

When warmed with a mixture of equal measures of alcohol and strong sulphuric acid, solanine dissolves with rose-red coloration. This reaction is stated to be characteristic.

As a micro-chemical test for solanine in plants, Schaarschmidt lays the section to be examined in moderately concentrated sulphuric or nitric acid, when in a few seconds the presence of the alkaloid is indicated by a beautiful rose colour. In this manner, Schaarschmidt recognised the presence of solanine in the tuber and stalk of *Solanum tuberosum*; and also in *S. nigrum*, *S. dulcamara*, *Capsicum annuum*, *Lycopersicum esculentum*, and *Mandragora officinalis*.

According to M. E. Wotczal (*Pharm. Jour.*, [3], xxi. 50), with the exception of strong sulphuric acid, only the two following tests are to be relied on for the detection of solanine:—

Mandelin's reagent, prepared by dissolving 1 part of ammonium meta-vanadate in 1000 parts of tri-hydrate of sulphuric acid ($H_2SO_4 + 2H_2O$). With solanine this gives a colour which is first yellow, changing to orange-red, purple-red, brown, pure red, violet, and blue-green, finally disappearing altogether. The reaction is very delicate.

Brandt's reagent, prepared by dissolving 0·3 gramme of sodium selenate in a mixture of 6 c.c. of strong sulphuric acid with 8 c.c. of water. If solanine be warmed with this reagent, the mixture, after cooling, becomes first violet-red, then orange-red and yellow-brown, the colour finally disappearing.

Solanine has the constitution of a glucoside, since, on boiling with dilute sulphuric or hydrochloric acid, it is hydrolysed with formation of a glucose and solanidine.

SOLANIDINE, $C_{26}H_{41}NO_2$,[1] according to Jorissen and Grosjean (abst. *Jour. Chem. Soc.*, 1891, p. 473), occurs ready-formed in the young sprouts of potatoes in the proportion of 1·5 per cent. It crystallises from alcohol or ether in long silky needles, but is thrown down on adding an alkali to one of its salts as a gelatinous precipitate (sometimes crystalline). Solanidine melts at 208° C. (191°, Firbas), and sublimes with partial decomposition. It is alkaline to litmus and has a sharp, bitter taste.

Solanidine is very slightly soluble in water, even when hot, but dissolves readily in strong alcohol, and is very easily soluble in ether. It is said to be extracted from its acidulated solutions by agitation with chloroform, and probably with ether also.

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[1] According to R. Firbas, solanidine contains $C_{40}H_{61}NO_2$, the formula for solanine being $C_{52}H_{93}NO_{18}$ (see footnote, page 74).

Solanidine forms salts which are mostly crystallisable, and sparingly soluble in water and acids. B,HCl forms rhombic prisms with end-faces, which may be sublimed, and are readily soluble in alcohol, but very sparingly soluble in water or in hydrochloric acid. B_2,H_2PtCl_6 is yellowish, amorphous, sparingly soluble in water, but readily soluble in acid.

Solanidine is unaltered by treatment with alkalies or dilute acids. It does not reduce either Fehling's solution or ammonio-nitrate of silver.

Solanidine dissolves in strong sulphuric acid with red colour, changing to dirty red, the base solanicine, $C_{26}H_{39}NO$, (?) being formed. With alcohol and sulphuric acid it behaves like solanine.

According to Jorissen and Grosjean, if a solution of solanidine in acetic acid be concentrated on the water-bath, hydrochloric acid and a little ferric chloride added, and the mixture then evaporated to dryness, a violet coloration is produced.

In 1883, the occurrence of poisonous symptoms in cattle, after feeding upon the potato-residues from a German distillery, led to an investigation by G. Kassner (*Archiv.*, 1885, p. 241). The residue was treated with ammonia, and then shaken with amylic alcohol, which, when separated and evaporated, left a crystalline residue consisting of solanine and solanidine, the latter predominating owing to the decomposition of the solanine under the influence of the acid mash. The presence of these poisonous alkaloids was attributed to the use either of sprouting or not perfectly ripe potatoes.[1]

GRANDIFLORINE is the name given by its discoverer, D. Freire (*Compt. rend.*, cv. 1074; abst. *Jour. Chem. Soc.*, lxvi. 166), to an alkaloid contained in the fruit of *Solanum grandiflora*, the "wolf-fruit" of Brazil. It gives precipitates with most of the general reagents for alkaloids; yields with strong sulphuric acid a bright yellow coloration, changing to red; with nitric acid a purplish-red colour; and with sulphuric acid and manganese dioxide a yellow colour, becoming first green and then violet. From the percentage of platinum contained in the chloroplatinate, the molecular weight of the alkaloid appears to be 236·4. Grandiflorine is insoluble in water, but soluble in alkalies and acids. Heated with caustic alkali, it evolves ammonia. It is very bitter, and acts as an energetic poison to sheep.

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[1] The occurrence and distribution of solanine in the potato, &c., and its relation to the growth of the plant, have been studied by M. E. Wotczal (*Pharm. Jour.*, [3], xxi. 50), and N. S. Klepsow (*Chem. Zeit. Rep.*, 1895, 338).

Aconite Alkaloids.[1] (*Addendum.*)

It has been recently shown that when aconitine is subj
hydrolysis by boiling with caustic alkali, it yields not onl
acid and aconine, as observed long since by C. R. A. W
but also a definite amount of acetic acid. Thus, on the
tion that the composition of crystallised aconitine is
represented by the formula $C_{33}H_{45}NO_{12}$, it is split up on hy
in the following manner :—

$$\underset{\text{Aconitine.}}{C_{33}H_{45}NO_{12}} + 2H_2O = \underset{\text{Acetic acid.}}{C_2H_4O_2} + \underset{\text{Benzoic acid.}}{C_7H_6O_2} + \underset{\text{Aconine.}}{C_{24}H_{39}NO}$$

Whether the above formula for aconitine is correct is qu
by eminent authorities, some of whom attribute to aconi
formula $C_{34}H_{47}NO_{11}$. If this formula be accepted, the
representing the saponification of aconitine becomes :—

$$C_{34}H_{47}NO_{11} + 2H_2O = C_2H_4O_2 + C_7H_6O_2 + C_{25}H_{41}NO$$

When the hydrolysis is conducted more cautiously, a
case when a salt of aconitine is heated with water only,
any addition of caustic alkali, acetic acid only is first
benzoyl-aconine, $C_{31}H_{43}NO_{11}$, being obtained simulta
This product proves to be identical with the picraconit
with in a single instance by T. B. Groves (Part ii.
which the formula $C_{31}H_{45}NO_{10}$ was attributed.

Pseudaconitine, the highly toxic alkaloid isolated by
Wright from the root of Nepaul aconite (*Aconitum fe*

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[1] Since the publication of Part ii. of this Volume, the chemist
stitution of the aconite alkaloids have formed the subject of seve
researches. The results of these investigations, except in some mi
are not in dispute ; but the claim to the discovery of the true co
aconitine has formed the subject of an embittered controversy, wh
degenerated into a discreditable wrangle, in which accusations o
bad faith have been freely made. Into the merits of this disp
necessary to enter, and in the above summary of recent rese
subject the author has intentionally omitted the mention of any

Those interested in the matter will find details in—

The Journal of the Chemical Society, lxi. 385, 395 ; lxiii.
lxv. 174, 176, 290 ; lxvii. 459 ;

Proceedings of the Chemical Society, 1894, pp. 6, 96 ;

The Pharmaceutical Journal, [3], xxiii. 86, 765 ; xxiv.
xxv. 575, 773, 1089, 1117 ;

The Chemist and Druggist, April 6th and 20th, 1895 ;

The British and Colonial Druggist, March 8th and May 17

Berichte der Deutsch. Gesell., 1894, pp. 27, 433, 664, 72
192, 1379, 2537 ;

Journ. für prakt. Chemie, xlv. 604, 606.

been found to behave similarly. Thus, when pseudaconitine sulphate is heated in a closed tube with water it splits off a molecular proportion of acetic acid, while a base of the constitution of veratryl-pseudaconine is simultaneously formed. On subsequently treating this body with caustic alkali, it suffers hydrolysis, and yields pseudaconine and veratric or dimethyl-protocatechuic acid.

These researches do not appear to have been extended as yet to the alkaloid of Japanese aconite; but this body, called by Paul and Kingzett *japaconitine*, and stated to have the formula $C_{66}H_{88}N_2O_{21}$, is now said to be identical with the alkaloid of *Aconitum napellus* (aconitine).

Titration of Alkaloids. (*Addendum.*)

Since the publication of Part ii. of this Volume, several important memoirs have appeared on the subject of the determination of alkaloids by titration with standard acid.[1] The general consensus of opinion is that the principle is of considerable practical value, but very discordant and disappointing results have been obtained in certain instances.

R. T. Thomson has investigated the general behaviour of indicators of neutrality in a very complete manner, and arranges the various indicators in three groups, to which the author has added Poirrier's soluble blue, CLB, as a fourth, thus:—

A. Methyl-orange Group.	B. Litmus Group.	C. Phenol-phthalein Group.	D. Poirrier's Soluble Blue.
Methyl-orange.	Litmus.	Phenol-phthalein.	Soluble blue, CLB
Cochineal.	Rosolic acid.	Turmeric.	
Congo-red.	Phenacetolin.		
Lacmoid.			
Iodeosin.			
Dimethyl-amido-azobenzene.			

For details of the behaviour of these various indicators, the author's paper on "Neutrality" should be consulted (*Analyst*, xvii. 186, 215; and *Pharm. Jour.*, [3], xxii. 752, 772).

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[1] The following are the chief contributions to the literature of the subject since Part ii. of this Volume was printed:—R. A. Cripps, *Pharm. Jour.*, [3], xxii. 511; A. H. Allen, *Pharm. Jour.*, [3], xxii. 752, 772; *Analyst*, xvii. 186, 215; C. Caspari, *Amer. Pharm. Review*, Nov. 1892; Caspari and Dohme, *Amer. Jour. Pharm.*, lxv. 473; Farr and Wright, *Pharm. Jour.*, [3] xxv. 124; L. F. Kebler, *Pharm. Jour.*, [3], xxv. 285; *Jour. Amer. Chem. Soc.*, xvii. 882; *Analyst*, Dec. 1895.

Broadly, the indicators of the methyl-orange group do not react with any but strong acids, but are sensitive to bases of feeble affinities, such as are many alkaloids. Litmus is often an uncertain indicator of neutrality, as the point at which the change of colour occurs does not in all cases correspond sharply with the formation of any definite salt. Phenol-phthalein is a very delicate and convenient indicator for the weakest acids (*e.g.*, hydrocyanic, carbonic, oleic) as well as the strongest, but it is absolutely indifferent to the great majority of the vegetable alkaloids, the midriatic bases, atropine, homatropine, hyoscine and hyoscyamine, and, according to Plugge, the volatile bases nicotine and conine, being the most notable exceptions. As a consequence of the indifference of the alkaloids to phenol-phthalein, their salts react with this indicator as if the acids were uncombined. This behaviour has been utilised by the author for the titration of quinidine hydriodide, and cinchonidine tartrate, which salts are obtained as precipitates in the ordinary process of separating cinchona-bases (compare Part ii. p. 460).

In titrating alkaloids much depends on the indicator employed and the method of applying it. Methyl-orange, rosolic acid, iodeosin, phloxin, phenol-phthalein, gallein, lacmoid, brazil-wood, logwood, litmus, and cochineal have all been employed and found advocates.

The behaviour of the alkaloids and organic bases with indicators of neutrality has been very imperfectly studied. It is frequently stated that a certain alkaloid is distinctly alkaline (presumably to litmus), but it is only rarely and of recent years that chemists appear to have attempted to estimate alkaloids by titration with standard acid. Where this is desired, phenol-phthalein is quite inapplicable, as already stated. Litmus answers in some cases, but by no means invariably With methyl-orange, in the majority of cases hitherto tried in the author's laboratory, a determination of tolerable accuracy and a fairly sharp end-reaction are obtainable.[1] Cochineal, brazil-wood, and logwood are often useful indicators.

In titrating quinine, an anomaly occurs which has misled more than one observer. This arises from the fact that the ordinary quinine sulphate of commerce, having, when anhydrous, the formula $(C_{20}H_{24}N_2O_2)_2,H_2SO_4$, though practically neutral to brazil-wood, logwood, and cochineal, is strongly alkaline to methyl-orange. The point of neutrality when titrating quinine with cochineal, brazil-wood, or logwood, is therefore reached when sufficient acid has been added to convert the quinine into the sparingly soluble sulphate of the formula Qu_2,H_2SO_4; whereas in the case of methyl-orange the end-reaction corresponds to the formation of the readily soluble *acid* sulphate of the formula Qu,H_2SO_4. Hence twice the volume of standard acid will be required by a given weight of quinine when methyl-orange is employed, as when brazil-wood, logwood, or cochineal is used as the indicator. If nitric acid or hydrochloric acid be substituted for sulphuric acid, the results are similar, the salts $Qu,2HNO_3$ and $Qu,2HCl$ being neutral to methyl-orange. The sparingly-soluble sulphate, Qu_2,H_2SO_4, is distinctly alkaline to litmus, and hence this indicator cannot be conveniently used for the titration of quinine, though the end-reaction is well marked. These observations may necessitate a revision of the accepted views on the basicity of quinine.[1]

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[1] This result, however, is not in accordance with the experience of L. F. Kebler (*Jour. Amer. Chem. Soc.*, xvii. 882).

Experiments made in the author's laboratory on the titration of cinchonine and cinchonidine with various indicators have led to such anomalous results as to render it doubtful if the constitution of these bases, or at least the composition of the commercial articles, is correctly understood.

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[1] The statements in the text are a record of facts observed independently in the author's laboratory, and carefully verified by numerous experiments. The main fact, however, was previously recorded by Seaton and Richmond (*Analyst*, xv. 43), who state that they "found that quinine bisulphate is neutral to methyl-orange, while the base itself has no action on phenolphthalein", and they based on these facts the following process for the determination of quinine in medicines :—To 25 c.c. of medicine add two drops of methyl-orange solution (0·25 gramme per litre) and two drops of phenolphthalein solution (0·5 gramme in 1 litre of proof spirit), and titrate with decinormal baryta solution until the free acid is all neutralised, as shown by the red colour just changing to a brown (*sic*). Note the volume of acid used, and continue the titration *slowly* until a pink colour appears, indicating the point of alkalinity. The difference between the two titrations, multiplied by the factor 0·0218, gives the weight of crystallised quinine sulphate (in grammes) in the 25 c.c. of medicine used. The above instructions assume that the medicine originally contained excess of acid, and was free from any inorganic base precipitable by baryta. Seaton and Richmond give figures showing that the method is fairly accurate when applied to pure sulphate of quinine, but in the opinion of the author the conditions under which the titrations are performed in practice render the method of little value. It is useless in presence of organic acids, and the colouring matter present in quinine wine prevents the end of the reaction from being accurately observed.

Owing to no formula for "quinine bisulphate" being given by Seaton and Richmond, nor the consequences pointed out, the fact on which their process was based was misinterpreted by the author (Part ii. page 403, footnote), and apparently has been quite overlooked by other writers.

The alkalimetric method of determining organic bases is often very valuable for the examination of an alkaloidal precipitate or residue, the purity of which is in doubt. C. Caspari asserts that the alkaloidal residues, obtained in the ordinary manner by gravimetric processes, invariably contain from 10 to 20 per cent. of impurity. This is probably an exaggeration, but the titration of a residue as a check on the weight is often very useful, and in some cases is more accurate than the gravimetric determination. Caspari recommends the solution of the residue in excess of decinormal hydrochloric acid, the solution being then titrated back with centinormal alkali, using brazil-wood as an indicator.

A valuable series of experiments on the titration of alkaloids as existing in pharmacopœial tinctures, in which several indicators were employed and compared, has been published by Farr and Wright (*Pharm. Jour.*, [3], xxv. 124). These chemists adopted in general the method of the author (Part ii. 131), modifying it to suit the characters of the indicator employed. The following are the details of their methods of working:—

1. Two gravimetric determinations were made by methods described in previous papers and the means taken.

2. The tincture was evaporated, the residual liquor rendered alkaline, and the alkaloid extracted with chloroform. Except in the cases of veratrum, lobelia, and colchicum, the alkaloid was once purified by shaking out with acidulated water, rendering the solution alkaline, and again extracting with chloroform. The chloroformic solution was then washed with a little distilled water before being titrated. When ammonia was used as a precipitant of the alkaloid, the washing was repeated until the water, on separation, ceased to become pink on addition of phenol-phthalein. The chloroformic solution thus obtained was utilised for the direct titration of the alkaloids with $\frac{N}{20}$ HCl; methyl-orange, iodeosin, and phloxin being used as indicators in the separate experiments. In using methyl-orange, a little distilled water was added along with two drops of the indicator: but in the case of iodeosin and phloxin a single drop of a $\frac{1}{1000}$ solution was found sufficient, and this was shaken up with the chloroformic solution until the latter was distinctly coloured. In the case of the methyl-orange, the termination of the reaction was indicated by the appearance of a light pink colour in the aqueous layer, while where iodeosin or phloxin has been used, the decolorisation of the chloroform marked the point of saturation.

3. The alkaloid obtained by the usual gravimetric process was

dissolved in a calculated excess of standard acid (usually 4 or 5 c.c.), the indicator added, followed by the addition of standard baryta-water until the neutral point was reached. These determinations were preferably made in the white porcelain dish in which the alkaloidal residue had been previously weighed. In this form of the process the indicators used were methyl-orange and brazil-wood, supplemented when possible by iodeosin and phloxin.[1] The end-reaction in the case of methyl-orange was indicated by the disappearance of the pink tint, and in that of brazil-wood by the production of a purple colour. When employing iodeosin or phloxin, except in direct titration, sufficient (neutral) ether was added to form a distinct supernatant layer after agitation. The acid was $\frac{N}{20}$ hydrochloric acid ($=1·825$ gramme of HCl per litre), and the alkali $\frac{N}{50}$ baryta-water ($=1·710$ gramme of BaH_2O_2 per litre). This solution, as pointed out by the author, possesses marked advantages over caustic soda.

The table on next page shows the results obtained by Farr and Wright by the foregoing methods. The last column has been added by the author, and represents the alkaloidal value of 1 c.c. of decinormal acid.

The results recorded in the table are considered by Farr and Wright to lead to the following conclusions:—

1. Volumetric methods are useless for the determination of the alkaloids of aconite, the large proportion of aconine present (equivalent ·02715) making the readings much too high.

2. They are also useless in the case of colchicum, which appears to contain a small percentage of alkaloid having definite basic properties, probably colchicine, with a considerable quantity of some other substance, which is chemically indifferent, but possibly equally active physiologically.[2]

---

[1] The indicators were prepared in the following manner:—

Methyl-Orange.—A tincture containing 1 grain of methyl-orange dissolved in a fluid ounce of proof spirit.

Brazil-Wood.—The U.S.P. test solution. This is made by boiling 50 grammes finely-cut brazil-wood with 100 c.c. distilled water for half an hour, replacing the water lost from time to time. The mixture is allowed to cool, the liquor strained off, water added to 100 c.c., and a further addition made of 25 c.c. of alcohol, and the whole filtered. In the author's laboratory, also, brazil-wood has been found a useful indicator of neutrality when titrating quinine.

Iodeosin and Phloxin.—An aqueous solution containing 1 part in 1000 measures.

[2] It appears more probable that colchicine, which is admittedly a very weak base, is incapable of accurate titration by any indicator, just as is the case with caffeine.

Table showing Comparative Results obtained in Determining Alkaloids in Tinctures (a) *Gravimetrically and* (b) *Volumetrically* (Farr and Wright).

Tincture.		Alkaloid by Weight.	Titration Result.							Alkaloidal Factor.
			By direct Titration of Chloroformic Solution with $\frac{HCl}{20}$.			By dissolving Crude Alkaloid in excess of $\frac{HCl}{20}$, and titrating back with $\frac{N}{50}$ baryta.				
			Methyl-Orange.	Iodeosin.	Phloxin.	Methyl-Orange.	Brazil-Wood.	Iodeosin.	Phloxin.	
Aconite,	1	·013	...	·018	·019	·020	...	·018	·018	·0647
	2	·014	·029	·019	·022	·022	·022	·022	·025	...
Belladonna,	1	·022	...	·022	·022	·022	·021	·020	·020	·0289
	2	·031	·033	·032	·034	·031	·032	·032	·030	...
Cinchona,	1	·074	...	·144	·145	·078	...	·072	·071	...
	2	·087	·093	087	·086	...	·052	...	...	...
Conium,	1	·047	...	·042	·042	·042	·042	·042	·042	·0127
	2	·024	·024	·024	·023	·024	·023	·024	·024	...
Colchicum,	1	·024	...	...	...	·005	...	...	·005	·0330
	2	028	...	...	...	·006	...	·005	...	...
Gelsemium,	1	·019	...	·018	·019	·019	·017	·018	·018	·0366
	2	·024	·024	·020	·024	·027	·024	·024	·024	—
Hyoscyamus,	1	·005	...	·005	·005	·005	·005	·005	·005	·0259
	2	·009	010	...	·0085	·008	·008	·008	·0086	...
Jaborandi,	1	·020	...	·018	·018	·017	·017	·017	·017	·0216
	2	·018	·018	·017	·017	·018	·018	·017	·018	...
Lobelia,	1	·009	...	·009	·008	·008	·008	·008	·008	·0285
	2	·009	·008	·009	·009	·007	·008	...	...	...
Nux Vomica,	1	·043	...	·044	·043	·043	·042	·041	...	·0364
	2	·066	·066	...	...	...	·066	·066	·065	...
Opium,[1]	1	·100	...	...	...	·096	·094	·095	·094	·0285
	2	·100	...	...	...	·099	·099	·097	·099	...
Stramonium,	1	·011	...	·011	·011	·011	·011	·010	·010	·0289
	2	·017	·017	...	...	·017	·017	·016	...	...
Veratrum Viride,	1	·023	...	·028	·022	·019	·020	·019	...	·0506
	2	·027	·027	·020	·023	·028	·021	·024	·019	...

---

[1] For the opium determinations a solution of anhydrous morphine obtained in estimating the tincture by the B.P. process was employed; this was dissolved in excess of $\frac{N}{20}$ HCl.

3. The factor for calculating the alkaloid in tincture of gelsemium was based on Spiegel's formula for gelsemine, $C_{22}H_{26}N_2O_3$.

4. The titration of the cinchona bases was attended with great difficulty, owing to the end-reaction being almost unobservable. With extreme care fairly accurate results were obtainable, but Farr and Wright's experience with tincture of cinchona was such as to cause them unhesitatingly to condemn the application of any volumetric process to the assay of this preparation. In several instances the results indicated by titration were exactly twice as great as those obtained by weighing.[1]

5. The volumetric determinations of the alkaloids in the tinctures of veratrum examined yielded results approximating very fairly to those of the gravimetric determination, but the formula weights of the alkaloids of veratrum differ so widely that such comparative accuracy could by no means be generally relied upon.

6. The tinctures which lend themselves most readily to determination by titration are those of belladonna, henbane, stramonium, conium, jaborandi, lobelia, nux vomica, and opium (for morphine).

As a general process for the volumetric assay of alkaloidal tinctures, Farr and Wright recommend the following as being in some cases almost as reliable and somewhat more expeditious than gravimetric methods:—"From 25–50 c.c. (in the case of hyoscyamus, 100 c.c.) of the tincture to be determined is introduced into a porcelain dish and evaporated over a water-bath, with addition of water if necessary, until all alcohol has been driven off. The residual extract is acidified and filtered through cotton wool into a stoppered separator, the dish and filter being washed with acidulated water, and the washings added to the contents of the separator. The liquid in the separator is then made alkaline and the alkaloids shaken out with three successive small quantities of chloroform. The chloroformic solutions are tapped off, and, when ammonia has been employed as the precipitant, washed with distilled water until the washings cease to give a pink colour with phenol-phthalein. A drop of $\frac{1}{1000}$ iodeosin solution is then added, and the whole well shaken until the chloroform is distinctly tinted. $\frac{N}{20}$ hydrochloric acid is then carefully run in from a burette graduated in tenths of a c.c., the mixture being well shaken after each addition of acid until the colour of the chloroform is discharged, when the volume of acid employed is observed, and the amount of alkaloid calculated therefrom by the employment of the appropriate factor. The process is not applicable to tincture of lobelia."

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[1] The factor employed by Farr and Wright (= ·0308 for 1 c.c. $\frac{N}{10}$ acid) was erroneous, and if applied to the experiments in which methyl-orange was used, ought to have resulted in figures double the true amount.

A general review of the methods of determining alkaloids has been published by L. F. Kebler (*Pharm. Jour.*, [3], xxv. 285), who strongly recommends alkalimetric titration, and gives tabulated results showing the results obtained by the application of the process to various tinctures, as compared with those yielded by gravimetric methods and by titration with Mayer's reagent. In a more recent paper (*Jour. Amer. Chem. Soc.*, 1895, xvii. 822), Kebler has published the results of numerous experiments on the determination of alkaloids by titration. In his opinion, the discordant results of different chemists are largely attributable to personal equation, each worker adopting a particular change of tint as the end-reaction. In Kebler's experiments, the titrations were made from acid to alkaline reaction, and the tints taken for the end-reactions were: Brazil-wood, from yellow to onion-red, the purple coloration first produced ultimately fading to an onion-red shade; cochineal, from yellow to bluish-red; hæmatoxylin, from yellow to brown-orange; litmus, from red to onion-red, and methyl-orange from red to straw-yellow. After testing the value of these indicators by titrating standard acid against standard alkali, the titration of commercially pure alkaloids was undertaken. In the case of quinine and codeine, 2 grammes of the alkaloid was dissolved in alcohol in a cylinder, and the solution made up to 100 c.c. with alcohol. To 10 c.c. of this, after the addition of the indicator, decinormal acid was added in slight excess, the liquid well agitated, and the excess of acid titrated back with decinormal alkali. Where the alkaloid was insoluble in alcohol, a weight of 2 grammes was warmed in a beaker on the water-bath with 75 c.c. of decinormal acid, until the alkaloid dissolved, the solution being then cooled and made up to 100 c.c. with water. Each 10 c.c. then contained 0·2 gramme of alkaloid and 7½ c.c. of decinormal acid solution. After adding the requisite amount of indicator to 10 c.c. and diluting to 50 c.c. the excess of acid was determined.[1]

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[1] Every precaution was taken in preparing the indicators, and the following quantities were used in each case:—Cochineal and litmus prepared as described in Sutton's *Volumetric Analysis*, of the former 5 drops, of the latter 10; phenolphthalein, 1 gramme per litre of 50 per cent. alcohol, 5 drops; hæmatoxylin, 1 gramme in 100 c.c. strong alcohol, 3 drops; brazil-wood solution, 5 drops; methyl-orange, 1 gramme in a litre of distilled water, 5 drops.

The results obtained by titrating pure alkaloids in the above manner were:—

Indicators.	Quinine.		Strychnine.	Morphine.	Codeine.
	La Wall.	Kebler.			
Brazil-wood, .	99·90	101·97	99·36	98·93	95·75
Cochineal, .	105·56	102·54	103·20	99·08	97·09
Hæmatoxylin, .	99·81	103·37	100·03	98·17	95·90
Litmus, . .	101·80	103·55	103·54	98·93	96·38
Methyl-orange, .	...	123·27[1]	104·21	100·59	98·11

The following table by Kebler shows the applicability of the process to crude alkaloids:—

Indicators.	Crude Morphine.		Crude Cocaine.
	La Wall.	Kebler.	
Brazil-wood, . . .	99·23	98·47	95·90
Cochineal, . . .	100·14	99·53	97·11
Hæmatoxylin, . .	99·08	97·59	95·74
Litmus, . . .	99·50	98·93	96·82
Methyl-orange, . .	102·10	100·02	100·14

With the same crude morphine the ash method gave 97·59 per cent., the lime-water method 98·22 per cent., and the absolute alcohol method 98·33 per cent. of pure morphine. The crude cocaine yielded, by the gravimetric method of E. R. Squibb (*Ephemeris*, iii. 1171), 97·3 per cent. of nearly pure cocaine.

For the extraction of alkaloids from their natural sources, Kebler employs a modification of Keller's process. To ten grammes of the dry drug in a 250 c.c. flask, 25 grammes of chloroform and 75 grammes of ether are added, and the flask well corked and shaken for some minutes. 10 grammes of 10 per cent. ammonia-water are then added, and the shaking continued at intervals for an hour. On adding 5 grammes more of the ammonia-water, the suspended powder coagulates, and the liquid can be poured off almost completely.

(1) 50 grammes weight of this liquid is evaporated on the water-bath, 10 c.c. of ether added, and the liquid again evaporated. The residue is dissolved in 15 c.c. hot alcohol, and water added to slight permanent turbidity. The indicator is then added, and an excess of the standard acid solution, which is titrated back with centinormal alkali.

(2) 50 grammes weight of the liquid is shaken with 20 c.c. of acidulated water in a separating funnel, the aqueous solution re-

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[1] It will be observed that the figure obtained by titrating quinine with methyl-orange is anomalous, but it does not approach 200, which is the result repeatedly approximately obtained in the author's laboratory. Possibly the discrepancy is due to a different colouring matter being employed by Kebler under the name of methyl-orange from that used by the author.

moved to a second separating funnel, and the shaking repeated twice more with 15 c.c. of slightly acidulated water. The acidulated water in the second funnel is made alkaline with ammonia, and the alkaloid removed by agitating successively with 20 c.c., 15 c.c., and 15 c.c. of a mixture of three parts (by volume) of chloroform with one of ether. The residue left on evaporation of the solvents is then treated as in process 1.

Nux vomica[1] and ipecacuanha root were treated by both the above processes; belladonna leaves by process 2 only. The results were as follow:—

	Per Cent. Alkaloids, Process 1.		Per Cent. Alkaloids, Process 2. Gravimetrically.		Per Cent. Alkaloids, Process 2. Volumetrically.	
	La Wall.	Kebler.	La Wall.	Kebler.	La Wall.	Kebler.
NUX VOMICA.						
Brazil-wood, . .	2·04	2·58	2·94	3·00	2·37	2·37
Cochineal, . .	2·64	2·69	2·86	3·10	2·42	2·39
Hæmatoxylin, .	2·18	2·24	2·88	3·11	2·23	2·27
Litmus, . .	2·38	2·34	2·93	3·05	2·55	2·37
Methyl-orange, .	3·02	3·64	2·93	3·02	2·65	2·61
IPECACUANHA.						
Brazil-wood, .	2·46	2·54	2·58	2·60	2·36	2·35
Cochineal, . .	2·59	2·49	2·63	2·68	2·52	2·33
Hæmatoxylin, .	2·48	2·54	2·58	2·68	2·35	2·33
Litmus, . .	2·55	2·57	2·62	2·60	2·40	2·25
Methyl-orange, .	2·95	3·30	2·66	2·63	2·89	2·61
BELLADONNA LEAVES.						
Brazil-wood, .	...	...	0·26	0·20	0·19	0·15
Cochineal, . .	...	...	0·28	0·20	0·24	0·14
Hæmatoxylin, .	...	...	0·27	0·22	0·21	0·18
Litmus, . .	...	...	0·24	0·18	0·20	0·15
Methyl-orange, .	...	...	0·25	0·20	0·23	0·20

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[1] The nux vomica, examined by the method of Dunstan and Short, showed 2·89 per cent. of crude alkaloid, and this, titrated with acid solution, yielded 2·12 per cent. of pure alkaloid. The figures in the text show that the Keller process produces an alkaloidal residue containing a larger percentage of pure alkaloid than that yielded by Dunstan and Short's method.

From the results of his experiments, Kebler concludes that methyl-orange is unsatisfactory with all strengths of acid, and that litmus, as ordinarily prepared, is also unsuitable. He finds hæmatoxylin, brazil-wood, and cochineal to give very promising results, the first being preferable, and cochineal the least manageable.

NON-BASIC VEGETABLE BITTER PRINCIPLES.

(*Appendix to Chapter on Alkaloids.*)

In addition to the alkaloids, there exist in plants a considerable number of bitter principles which are not possessed of basic properties. Some of these have more or less well-defined acid characters, while others are wholly indifferent. Not a few have the constitution of glucosides; in fact, the whole group can be conveniently divided into glucosides and non-glucosidal bitters. Only a few of the leading members of each class require consideration here.

GLUCOSIDES.

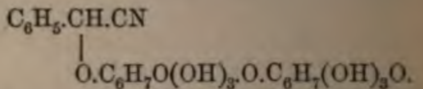
The name glucoside is applied to numerous bodies possessing the common property of yielding glucose, or an analogous body of the sugar-group, as one of the products of their hydrolysis on treatment with a dilute acid. Thus *salicin*, which is a typical glucoside, when boiled with dilute sulphuric acid is hydrolysed, with formation of glucose and the alcohol-like body saligenol or saligenin,

$$\underset{\text{Salicin.}}{C_{13}H_{18}O_7} + H_2O = \underset{\text{Dextrose.}}{C_6H_{12}O_6} + \underset{\text{Saligenol.}}{C_7H_7O.OH}.$$

Some of the glucosides yield ordinary dextrose on hydrolysis, but certain members of the class yield unfermentable or optically inactive glucoses, and a few yield bodies which, though carbohydrates, are not of the nature of true sugars. Usually, only one species of sugar results from the decomposition of a homogeneous glucoside, but in a few instances two distinct kinds of sugar are formed.

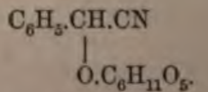
Although the great majority of the glucosides at present known are natural products of the vegetable kingdom, some few bodies of animal origin belong to the same class.[1] Among these is the *cerebrin* from brain-tissue, which on hydrolysis yields a sugar isomeric with galactose. The protein-like bodies *mucin* and *condylin*, occurring in mucus and cartilage respectively, also yield a glucose on hydrolysis. Another body, obtained from excrementious matter and Purrée or Indian yellow, is also of a glucosidal nature, consisting as it does of the magnesium salt of *euxanthic acid*, which splits up on boiling with dilute acid into euxanthone and glycuronic acid:—$C_{19}H_{18}O_{11} = C_{13}H_8O_4 + C_6H_{10}O_7$. Glycuronic acid, $COH(CH.OH)_4.COOH$, may be regarded as glucose in which one of the $CH_2.OH$ groups has been oxidised to a carboxyl group (Allen's *Chemistry of Urine*, p. 37).

A recent research by E. Fischer (*Ber.*, xxviii. 1511) on *amygdalin*, the glucoside of bitter almonds, is very suggestive of future discoveries in the same direction. In has been long known that amygdalin splits up, under the influence of emulsin, into benzoic aldehyde (oil of almonds), hydrocyanic acid, and glucose. These facts, and the conversion of the glucoside into mandelic and amygdalic acid, caused Schiff to regard it as a compound of benzaldehyde-cyanhydrin with a disaccharid, having the following structural formula:—



$$\begin{array}{l} C_6H_5.CH.CN \\ \qquad\quad | \\ \qquad\quad O.C_6H_7O(OH)_3.O.C_6H_7(OH)_3O. \end{array}$$

Fischer, however, regards the interpretation of the constitution of the saccharine residue as incorrect or incomplete. He considers that amygdalin is a derivative of maltose, or of a similarly constituted disaccharid. This opinion is supported by the fact that, by the aid of the yeast-ferment, half the sugar can be split off as glucose without the nitrogenous part of the molecule being at all affected. A new glucoside, very similar to amygdalin, is thus produced, having the formula:—



$$\begin{array}{l} C_6H_5.CH.CN \\ \qquad\quad | \\ \qquad\quad O.C_6H_{11}O_5. \end{array}$$

Fischer calls this body "amygdonitril glucoside." It closely resembles amygdalin in chemical behaviour, but differs very much in physical properties. Emulsin rapidly decomposes it into benzoic aldehyde, hydrocyanic acid, and one molecule of glucose.

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[1] The "*indican*" isolated from the urine of herbivorous animals was at first considered to be identical with the vegetable glucoside of the same name; but further investigation has shown that, although it yields indigo, no glucose results from its hydrolysis, and that it is the potassium salt of indoxyl-sulphuric acid, $C_8H_6N.SO_4H$.

Certain of the natural glucosides have been prepared synthetically. Thus Michael prepared formyl-phenylglucoside or helicin by the action of aceto-chlorhydrose on the potassium-derivative of salicylic aldehyde. From helicin, by the action of nascent hydrogen, he obtained salicin, and by fusing this with benzoic anhydride populin was obtained.

The hydrolysis of the glucosides may be effected in a few instances by simply boiling the compound with water, or by heating the aqueous solution under pressure; but the change proceeds much more rapidly in presence of an acid. Heating with caustic alkali, or, preferably, baryta-water, may be employed in some cases; but salicin and some other glucosides are wholly unchanged when treated with alkalies.

Hlasewetz classifies the glucosides in five series as follows:—

1. *Glucosides proper*, which yield on hydrolysis one or two molecules of a glucose. This group includes the great majority of the natural glucosides.

2. *Phloroglucides*, which yield phloroglucol instead of glucose on hydrolysis; such as maclurin, phloretin, and quercitin.

3. *Phloroglucosides*, yielding both phloroglucol and a sugar on hydrolysis; such as phloridzin, quercitrin, robinin, and rutin.

4. *Mannides*, or compounds with a mannitol derivative other than glucose.

5. *Nitrogenised glucosides;* such as amygdalin, chitin, indican, myronic acid, and solanine.

Many tannins, phlobaphenes, and allied bodies appear to be related to the glucosides, but their amorphous character and the slowness with which they undergo hydrolysis suggest that they are derivatives of *anhydrides* of mannitol and of the glucoses, such as, for instance, dextrin. This view is quite consistent with the production of glucose by boiling with dilute acids, the glucose so formed being a secondary product.

The non-carbohydrate products of the hydrolysis of the glucosides are of a very diverse nature, including hydrocarbons, alcohols, aldehydes, acids, thiocarbimides, &c.

Hydrolysis of the glucosides often takes place through the agency of certain peculiar unorganised ferments occurring in the plant together with the glucoside. The emulsin of bitter almonds and the myrosin of mustard are examples of these. These unorganised ferments have a very limited power of effecting such decompositions, their influence being exerted only on a few glucosides of closely related composition. Emulsin, however, not only decomposes amygdalin, the glucoside with which it is associated in nature, but also salicin (but not populin), æsculin, and many others.

As a rule, the glucosides are best isolated by extracting the substance with water or dilute alcohol, clarifying the solution by lead acetate, removing the excess of lead from the filtrate by sulphuretted hydrogen, and evaporating the filtered solution to the crystallising point. A useful method of separating some of the glucosides is to precipitate the solution with alum and a slight excess of ammonia, when the glucoside is thrown down in combination with alumina as a species of lake, which may be filtered off and decomposed by an acid.

One or two basic plant-principles (*e.g.*, solanine) have a glucosidal constitution, but the great majority of the glucosides are indifferent or feebly acid bodies. Most of them are soluble in water and alcohol, but insoluble, or nearly so, in ether. A convenient solvent is a mixture of chloroform and ether. Such immiscible solvents as dissolve them extract the glucosides from their acidulated aqueous solutions, a behaviour which affords a general method of separating the majority of the glucosides from the stronger alkaloids, but not from weak bases like narcotine, caffeine, &c. (Compare Part ii. pages 158, 159.)

The great majority of the natural glucosides have a *bitter taste*. The remarkable exception to this rule apparently afforded by glycyrrhizin, the intensely *sweet* principle of liquorice-root (the underground stem of *Glycyrrhiza glabra*), has been explained by the researches of Habermann (*Ber.*, x. 870) and Sestini (*Pharm. Jour.*, [3], x. 327), which render it very doubtful if glycyrrhizin is a true glucoside.[1] Habermann states (*Annalen*,

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[1] GLYCYRRHIZIN or GLYCYRRHIZIC ACID, $C_{44}H_{63}NO_{18}$, occurs in liquorice root in the form of a calcium or ammonium salt. As obtained by decomposing the insoluble lead salt with sulphuretted hydrogen, glycyrrhizic acid is an amorphous substance resembling albumin, which turns brown at 100°, and at a higher temperature swells up and carbonises. It gelatinises with cold water, but dissolves readily on heating. The solution is intensely sweet, is acid to litmus, and decomposes calcium and barium carbonates on boiling. Glycyrrhizic acid is tribasic, and forms soluble salts of an extremely sweet taste. It dissolves freely in dilute spirit, but is only sparingly soluble in absolute alcohol.

Glycyrrhizin is alleged to have 140 times the sweetening power of cane-sugar, and hence liquorice has been employed for sweetening malt liquors, especially stout. For the detection of liquorice in beer, &c., R. Kayser (abst. *Analyst*, x. 125) proposes the following process, which he states will permit the detection of less than 1 gramme of liquorice in 1 litre of beer :—1 litre of the sample is cxcvii. 105; abst. *Jour. Chem. Soc.*, xxxviii. 671) that by boiling with dilute acids glycyrrhizin yields glycyrrhetin (a crystallised nitrogenous compound of the formula $C_{32}H_{47}NO_4$) and parasaccharic acid, $C_6H_{10}O_8$, isomeric with ordinary saccharic acid. This acid is a brown gum, soluble in water and alcohol, which yields uncrystallisable salts (distinction from saccharic acid) and reduces Fehling's solution.

Many of the glucosides are optically active, being, as a rule, *lævo*-rotatory, although on hydrolysis they yield a *dextro*-rotatory glucose. Some of them (*e.g.*, digitalin, saponin, strophanthin) are intensely poisonous.

Such of the glucosides as are decomposed by alkalies with formation of a reducing sugar reduce Fehling's solution on boiling, and some reduce ammonio-silver nitrate. A few glucosides are precipitated by tannin, picric acid, and some other alkaloidal reagents. Some glucosides (*e.g.*, saponin, strophanthin) yield aqueous solutions which froth strongly on agitation, while the solutions of others coagulate on heating.

Some of the glucosides give characteristic colour-reactions with acids, &c.; but the chemical reactions of the majority have been very incompletely studied.

evaporated on a water-bath to half its volume, and on cooling precipitated with a slight excess of a concentrated solution of lead acetate. After standing twenty-four hours, the precipitate is filtered off, well washed, and rinsed into a flask, and sufficient water added to bring the whole to about 350 c.c. The liquid is then heated for an hour on the water-bath, and sulphuretted hydrogen passed into it while still warm. The liquid is allowed to become cold, well agitated, passed through a folded filter, and the sulphuretted hydrogen washed out. The lead sulphide remaining on the filter is said to retain the glycyrrhizic acid of the liquorice. It is rinsed into a flask with about 150 c.c. of proof-spirit, the liquid heated to boiling, and filtered. The filtrate is reduced by evaporation to a few c.c., and a few drops of ammonia added, which will turn the pale yellow liquid brown-yellow. This is then evaporated to dryness, the residue dissolved in 2 to 3 c.c. of water, and the liquid filtered. The filtrate will possess the characteristic taste of liquorice, and when heated on the water-bath with a few drops of hydrochloric acid will yield a flocculent resinous mass of glycyrrhetin. In the absence of liquorice, the residue will be tasteless or slightly bitter, and will give at most only a whitish turbidity on heating with hydrochloric acid.

The composition and adulterations of commercial liquorice or Spanish juice have been discussed by B. Dyer (*Analyst*, xiii. 124). Analyses of liquorice-root have been published by F. Sestini (abst. *Jour. Chem. Soc.*, 1878, p. 740) and by J. W. Nickum (*Amer. Jour. Pharm.*, June 1895).

The "Compound liquorice powder" of the British Pharmacopœia ought to contain 8·33 per cent. of sulphur, but this ingredient is not unfrequently omitted partially or entirely.

The following is a tabular list of the better-known glucosides. members of the class are constantly being discovered.

Name.	Chief Source.	Formula.	M. Pt. °C.	Products of Hydrolysis.	Other Characters.
Acorin,	Calamus root,	$C_{36}H_{60}O_6$	..	Glucose, $C_6H_{12}O_6$ Calamus oil, $C_{10}H_{16}$	Bitter.
Æsculin,	Horse-chestnut bark.	$C_{15}H_{16}O_9$	197	Glucose, $C_6H_{12}O_6$ Æsculetin, $C_7H_6O_4$	Solutions highly fluorescent. Page 23.
Amygdalin,	Bitter almonds,	$C_{20}H_{27}NO_{11}$	200	Dextrose, $C_6H_{12}O_6$ Hydrocyanic acid, CHN Benzaldehyde, C_7H_6O	Slightly bitter; neutral; not poisonous. Page 91.
Antiarin,	Arrow poison, (*Antiaris toxicaria*),	$C_{14}H_{20}O_5+2aq$,	221	Reducing sugar Resinous substance	Colourless shining plates. Neutral. Heart poison. Page 141, footnote.
Apiin,	Parsley; celery,	$C_{27}H_{32}O_{16}$	228	Glucose, $C_6H_{12}O_6$ Apigenin, $C_{15}H_{10}O_5$	Aqueous sol. gelatinises; alkaline sol. light yellow.
Arbutin,	Leaves of red bearberry, &c.,	$C_{12}H_{16}O_7$	187·5	Glucose, $C_6H_{12}O_6$ Quinol, $C_6H_6O_2$	Silky needles. Does not reduce Fehling's solution.
Bornesite,	Borneo caoutchouc,	$C_7H_{14}O_6$	175	Dambose, $C_6H_{12}O_6$ Methyl-alcohol, CH_4O	Rhombic prisms.
Caincin,	Root of *Chiococca anguifuga* and *C. racemosa*,	$C_{40}H_{64}O_{18}$	..	Glucose, $C_6H_{12}O_6$ Caincetin, $C_{22}H_{34}O_3$	Feeble acid; bitter astringent taste. Slender needles.
Carminic acid,	Cochineal,	$C_{17}H_{18}O_{10}$	..	$C_6H_{10}O_5$, inactive Carmine red, $C_{11}H_{12}O_7$	Dark red powder. Sol. in water or alcohol. See Cochineal, Part i. page 363.
Chitin,	Elytra of beetles; carapaces of crustacea,	$C_{15}H_{26}N_2O_{10}$	..	Glycosamin, $C_6H_{13}NO_5$ Acetic acid, $C_2H_4O_2$	Insol. in most solvents. Amorphous.
Coniferin,	Sap of conifers; beetroot and asparagus,	$C_{16}H_{22}O_8$	185	Glucose, $C_6H_{12}O_6$ Coniferyl alcohol, $C_{10}H_{12}O_3$	Crystalline; bitter, does not reduce Fehling's solution, p. 97.
Convolvulin,	Jalap-root,	$C_{31}H_{50}O_{16}$	150	Glucose, $C_6H_{12}O_6$ Convolvulinol, $C_{13}H_{21}O_3$	Colourless, brittle. Red with H_2SO_4. Page 146.

Name.	Chief Source.	Formula.	M. Pt. °C.	Products of Hydrolysis.	Other Characters.
Dambonite,	Borneo caoutchouc,	$C_8H_{16}O_6$	190	Dambose, $C_6H_{12}O_6$ Methyl-alcohol, CH_4O	Prisms. Not fermentable. Does not reduce Fehling's solution.
Daphnin,	Bark of *Daphne mezereum*, &c.,	$C_{15}H_{10}O_9$	200	Glucose, $C_6H_{12}O_6$ Daphnetin, $C_6H_2(OH)_2O_2.C_3H_3O_2$	Bitter, astringent, rectangular prisms. Yellow solution in alkalies. Slowly reduces Fehling's solution.
Digitalin,	Foxglove (*Digitalis purpurea*).	$C_{29}H_{46}O_{12}$	..	Glucose, $C_6H_{12}O_6$ Digitalose, $C_7H_{14}O_5$ Digitaligenin, $C_{16}H_{22}O_2$	Page 131.
Digitonin,	Foxglove,	$C_{27}H_{46}O_{14}$	235	Glucose, $C_6H_{12}O_6$ Galactose, $C_6H_{12}O_6$ Digitogenin, $C_{15}H_{24}O_3$	Page 133.
Frangulin,	Bark, &c., of *Rhamnus Frangula*,	$C_{21}H_{20}O_9$	226	Rhamnose, $C_6H_{12}O_5$ Emodin, $C_{15}H_{10}O_5$	Yellow, crystalline. Sol. in alkalies, with intense red colour.
Helleborin,	With *Helleboreïn* in root of *Helleborus niger*, &c.,	$C_{36}H_{42}O_6$	..	Glucose, $C_6H_{12}O_6$ Helleboresin	Crystalline ; bitter. Page 68.
Hesperidin,	Unripe oranges, &c.,	$C_{22}H_{26}O_{12}$	251	Glucose, $C_6H_{12}O_6$ Hesperetin, $C_{16}H_{14}O_6$	Crystalline, tasteless, insoluble. Does not reduce Fehling's sol. Evaporated with KHO, gives red residue, turned violet by H_2SO_4.
Indican,	Woad (*Isatis tinctoria*) and indigo,	$C_{26}H_{31}NO_{17}$	..	Indiglucin (Dextrose), $C_6H_{12}O_6$ Indigotin, C_8H_5NO	Part i. page 292.
Jalapin (or Scammonin),	Jalap (or Scammony),	$C_{34}H_{56}O_{16}$	150	Glucose, $C_6H_{12}O_6$ Jalapinol, $C_{32}H_{62}O_7$	Amorphous resin. Page 146.
Morindin,	Morinda citrifolia, &c.,	$C_{26}H_{28}O_{14}$	245	Glucose, $C_6H_{12}O_6$ Morindon, $C_{15}H_{10}O_5$	Yellow needles. Alkali solution orange-red.
Myronic acid,	Black mustard and rape seeds,	$C_{10}H_{19}NS_2O_{10}$	..	Dextroglucose, $C_6H_{12}O_6$ Sulphuric acid, H_2SO_4 Allyl thiocarbimide, $C_3H_5.NCS$	Page 105.
Phloridzin,	Root-bark of apple, pear, plum, &c.,	$C_{21}H_{24}O_{10}$	109	Dextroglucose, $C_6H_{12}O_6$ Phloretin, $C_{15}H_{14}O_5$	Silky, bitter needles, soluble in water.

Name.	Chief Source.	Formula.	M. Pt. °C.	Products of Hydrolysis.	Other Characters.
Picrocrocin,	Saffron,	$C_{38}H_{66}O_{17}$	..	Crocose, $C_6H_{12}O_6$ Terpene, $C_{10}H_{16}$	Fine prisms; bitter. Part i. page 349.
Populin,	Aspen poplar, &c.,	$C_{20}H_{22}O_8$	180	Dextrose, $C_6H_{12}O_6$ Benzoic acid, $C_7H_6O_2$ Saligenol, $C_7H_8O_2$	Page 100.
Quercitrin,	Quercitron,	$C_{36}H_{38}O_{20}$	168	Isodulcite, $C_6H_{14}O_6$ Quercetin, $C_{24}H_{16}O_{11}$	Yellow needles. Part i. page 340.
Quinovin,	Cinchona and allied barks,	$C_{38}H_{62}O_{11}$	..	Quinovite, $C_6H_{12}O_4$ Quinovic acid, $C_{32}H_{48}O_6$	Crystalline, bitter powder. Part ii. page 443.
Ruberythric acid,	Madder-root,	$C_{26}H_{28}O_{14}$	..	Dextroglucose, $C_6H_{12}O_6$ Alizarin, $C_{14}H_8O_4$	Yellow silky prisms. Part i. page 264.
Rutin,	Rue, buckwheat, capers, &c.,	$C_{42}H_{50}O_{25}$	..	Isodulcite, $C_6H_{14}O_6$ Quercetin, $C_{24}H_{16}O_{11}$	Pale yellow needles, sol. boiling water or alcohol. Part i. page 341.
Salicin,	Willow or poplar bark, &c.,	$C_{13}H_{18}O_7$	201	Glucose, $C_6H_{12}O_6$ Saligenol, $C_7H_8O_2$	Page 98.
Sinalbin,	White mustard seeds,	$C_{30}H_{44}N_2S_2O_{16}$	..	Dextroglucose, $C_6H_{12}O_6$ Sinapine sulphate, $C_{16}H_{23}NO_5.H_2SO_4$ Acrinyl thiocarbimide, $C_7H_7O.NCS$	Page 103.
Saponins,	Soapwort,	$C_nH_{2n-8}O_{10}$	..	Glucose, $C_6H_{12}O_6$ Sapogenin, &c.	Page 123.
Strophanthin,	Strophanthus,	$C_{31}H_{48}O_{12}$ (?)	173	Strophantidin Glucose, $C_6H_{12}O_6$	Page 139.
Tannins,	Various,	..	..	Various.	Part i. page 79.
Urechitin,	Apocyne Urechitis-suberecta,	$C_{28}H_{42}O_8$	..	..	Very poisonous; resembles strophanthin.

ly two reactions can be regarded as applying to nearly every er of the class of glucosides, namely:—the hydrolysis by treatwith dilute acids, with production of a reducing sugar, and itation by a solution of ammonium molybdate slightly aciduwith hydrochloric acid.

ucosides of Conifers.

NIFERIN, $C_{16}H_{22}O_8$, is a glucoside occurring in the cambium f coniferous trees, and found also in beetroot and asparagus. readily prepared by evaporating the previously boiled and d juice to the crystallising point. It forms white satiny es, often arranged in stellate groups, which contain $2H_2O$ and esce in dry air, become anhydrous at 100°, and melt at 185°. erin is soluble in about 200 parts of cold water, but more ly in hot water and in alcohol. It is insoluble in ether. aqueous solution of coniferin has a bitter taste, and is lævoory ($[\alpha]_D = -66{\cdot}9°$ at 20° C.). Coniferin dissolves in strong uric acid with red colour, a deep blue resin separating on ion with water. Moistened with phenol, and then treated concentrated sulphuric or hydrochloric acid, coniferin rapidly res a deep blue colour, the change occurring in sunlight st instantaneously. By this reaction coniferin can be readily ted in pine wood, and, conversely, pine wood moistened with chloric acid may be used to detect phenols.

niferin gives no reactions with metallic solutions, and does educe Fehling's solution. Chromic acid mixture oxidises vanillin (compare Part i. page 62). Treated with emulit is gradually hydrolysed into glucose and coniferyl hol:—

$$(CH_3O).C_6H_3(C_3H_4.OH)O.C_6H_{11}O_5+H_2O=$$
$$(CH_3O).C_6H_3(C_3H_4.OH).OH+C_6H_{12}O_6.$$

lute acid be used as the hydrolysing agent instead of in, the coniferyl alcohol becomes polymerised to a resinoid

INGIN, $C_{17}H_{24}O_9+H_2O$, has the constitution of a hydrate hydroxymethyl-coniferin (see page 123),

$$(CH_3O)_2C_6H_2(C_3H_4OH)O.C_6H_{11}O_5+H_2O.$$

ucosides of Willow and Poplar.

interesting glucoside salicin occurs naturally in the nd leaves of the willow, with populin in the poplar, and in many other species of *Salix*. Salicin is likewise present in casto eum and meadow-sweet (*Spiræa ulmaria*).

Salicin, $C_{13}H_{18}O_7$, or $C_6H_4(O.C_6H_{11}O_5).CH_2.OH$, has the consti tution of an ortho-hydroxybenzyl glucoside. It has bee prepared synthetically, and also results from the action of nascen hydrogen on helicin, $C_{13}H_{16}O_7$, or by the saponification o populin by boiling baryta-water:—

$$\underset{\text{Populin.}}{C_{20}H_{22}O_8} + H_2O = \underset{\text{Benzoic acid.}}{C_7H_6O_2} + \underset{\text{Salicin.}}{C_{13}H_{18}O_7}.$$

For the preparation of salicin, willow-bark[1] should be exhauste with boiling water, the decoction concentrated, digested for som time with litharge, and the filtered liquid concentrated to a syru and left to crystallise. The impure salicin is recrystallised fro alcohol, and may be decolorised by animal charcoal. Salicin form a crystalline, silky powder, or white shining, tabular crystals c scales, melting at 198°–201° C., and recrystallising if allowe to cool. If more strongly heated, salicin decomposes at abou 240° with evolution of acid vapours and formation of glucos and saliretin.[2]

Salicin has a very bitter taste, and possesses febrifugal prope ties.[3] It dissolves sparingly in cold (1 : 28), but very readily i hot water (1 : 0·7), and is also soluble in alcohol (1 : 30, cold 1 : 2, boiling); but is nearly insoluble in ether, and wholly so i chloroform, carbon disulphide, turpentine, and most other solvent immiscible with water, except amylic alcohol, which is stated b Dragendorff to extract salicin from aqueous liquids. Solutions o salicin are bitter, neutral to litmus, and lævo-rotatory. $[\alpha]_D =$ $-55·8°$.

With strong sulphuric acid, solid salicin yields a bright re coloration, destroyed on addition of water with deposition of a powder of deep red colour, insoluble in water or alcohol.

Salicin dissolves readily and completely in cold hydrochlori acid of 1·11 sp. gr., but on warming the solution a voluminous white precipitate of saliretin[1] separates long before the boiling-point is reached.

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[1] Willow-bark is generally stated to contain about 4 per cent. of salicin Gessler found in ten samples from 1·06 to 3·13 per cent., the usual proportio lying between 2·2 and 2·5 per cent.

[2] If the brown residue (avoiding over-heating) be heated with water, th solution will give a violet coloration with ferric chloride.

[3] Salicin is official in the British and United States Pharmacopœia It is used in doses of 3 to 30 grains, in cases of acute rheumatism, inte mittent fever, &c., and was formerly employed as an adulterant of quinine When taken internally, a portion is excreted unchanged, while the re mainder appears in the urine as salicylic acid, salicylic aldehyde, an saligenol.

Salicin dissolves in a 10 per cent. solution of caustic potash or soda. The solution reduces Fehling's solution on boiling.

With Fröhde's reagent, salicin gives almost immediately a fine purple coloration.

If salicin be dissolved in nitric acid, the solution evaporated at 100°, and the residue re-dissolved in a little water, a yellow solution is obtained, owing to the formation of nitro-salicylic acid. If this solution be again evaporated, and ferric chloride added to the residue, a red coloration will be produced.

If a small quantity of salicin be heated with a little bichromate of potassium, a few drops of sulphuric acid and some water added, and the mixture heated, vapours of salicylic aldehyde, $C_6H_4(OH).COH$, having the odour of meadow-sweet, will be evolved.

Salicin is not precipitated by tannin, gelatin, neutral or (except in very concentrated solution) basic lead acetate, and gives no reaction with Mayer's solution or other of the general reagents for alkaloids.

Salicin is quite unaffected by treatment with alkalies, even when boiling, but when warmed with dilute hydrochloric or sulphuric acid, it is hydrolysed with formation of dextrose and saligenin; a portion of the latter product undergoing dehydration with formation of saliretin.

SALIGENIN or SALIGENOL, $C_7H_8O_2$; *i.e.*, $C_6H_4(OH).CH_2.OH$, bears the same relation to salicylic acid that ethylic alcohol bears to acetic acid, and hence has the constitution of ortho-hydroxybenzyl alcohol. It has been obtained by the action of sodium amalgam and water on salicylic aldehyde, and by heating phenol with methylene chloride and caustic soda. It crystallises in small tables, which melt at 82° and sublime at 100°. It dissolves in about 15 parts of cold water, in almost all proportions of boiling water, and is also soluble in alcohol, ether, and benzene. It may be crystallised from the last solvent, and extracted from its aqueous solutions by ether. The aqueous solution of saligenol is coloured indigo-blue by ferric chloride. The solid substance dissolves with bluish-red coloration in strong sulphuric acid.

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[1] SALIRETIN. $C_{14}H_{14}O_3$; *i.e.* $C_6H_4(OH).CH_2.O.C_6H_4(CH_2.OH)$. Saliretin of the above formula is obtained by heating salicin to 80° C. with 10 parts of fuming hydrochloric acid, treating the product with water, dissolving the precipitate in dilute alcohol, and precipitating again by addition of brine.

Saliretin is a yellowish resin soluble in alkalies. It is extracted from acidulated solutions by agitation with ether. It does not yield either salicylic acid or aldehyde when treated with oxidising agents. Strong nitric acid converts it into picric acid. With sulphuric acid, saliretin behaves like salicin.

Saliretin differs from a double molecule of saligenin by the elements of water:—$2C_7H_8O_2 - H_2O = C_{14}H_{14}O_3$. By the action of concentrated sulphuric acid on salicin an anhydride of the formula $C_{28}H_{26}O_5$ is obtained.

Saligenol is best obtained by acting on salicin in aqueous solution by the ferment emulsin, obtained by macerating pressed almonds with cold water for some hours, and precipitating the solution with alcohol.

Caffeol, $C_8H_{10}O_2$, the substance to which the aroma of roasted coffee is due, appears to have the constitution of a methyl-ether of saligenol, and the formula $C_6H_4(OH).CH_2O(CH_3)$. (Compare Part ii. page 532.)

BENZOYL-SALICIN or POPULIN, $C_6H_4(CH_3OH).O.C_6H_{10}BzO_5$ or $C_{20}H_{22}O_8+2H_2O$, occurs in the bark and leaves of the aspen-poplar, and has been obtained synthetically by fusing salicin with benzoic anhydride. It forms delicate needles, which become anhydrous at 100° and melt at 180°. Populin is soluble in 2420 parts of cold or in 42 of boiling water. Its taste resembles that of liquorice. It is coloured bright red by strong sulphuric acid, and purple by Fröhde's reagent. By boiling with dilute acids populin is converted into benzoic acid, dextrose, and saliretin. Boiled with baryta-water it yields benzoic acid and salicin, but is not hydrolysed by emulsin.

Glucosides of Mustard.

Black and white mustard, the seeds of *Brassica* or *Sinapis nigra* and *S. alba*, present very close resemblances in general composition. Thus they both contain a *fixed oil*, allied to rape oil, and in both seeds there is a considerable proportion of *albuminous matters* and *mucilage*, but starch is absent. Both seeds contain the soluble ferment *myrosin*, the white mustard usually containing the larger proportion. Both kinds of mustard also contain *sinapine thiocyanate*, $C_{16}H_{24}NO_5.CNS$, but the proportion in the white seeds is much larger than in the black. In addition to the above-named bodies, common to the two kinds of mustard, each kind of seed contains a *glucoside* peculiar to itself. The glucoside of white mustard is preferably called *sinalbin*; but has also been described under the names of sinapin, sinapine sulphocyanide, and sulpho-sinapisin. The glucoside of black mustard has been termed sin-nigrin or *sinigrin*, but is more frequently alluded to as *myronic acid*, which exists in the seed as a potassium salt. Under the influence of the nitrogenised ferment myrosin, both sinalbin and sinigrin are decomposed, glucose being formed in each case. The other products of the action of myrosin on sinalbin are sinapine sulphate and acrinyl (*p.*-hydroxy-benzyl) iso-thiocyanate or thiocarbimide, while myronic acid under similar treatment yields sulphuric acid and allyl iso-thiocyanate or thiocarbimide (mustard oil). The following equations represent the reaction in both cases:—

WHITE MUSTARD:—

$$\underset{\text{Sinalbin.}}{C_{30}H_{44}N_2S_2O_{16}} = \underset{\text{Glucose.}}{C_6H_{12}O_6} + \underset{\text{Sinapine sulphate.}}{C_{16}H_{23}NO_5,H_2SO_4} + \underset{\substack{\text{Acrinyl} \\ \text{thiocarbimide.}}}{C_7H_7O,NCS}$$

BLACK MUSTARD:—

$$\underset{\text{Myronic acid.}}{C_{10}H_{19}NS_2O_{10}} = \underset{\text{Glucose.}}{C_6H_{12}O_6} + \underset{\substack{\text{Sulphuric} \\ \text{acid.}}}{H_2SO_4} + \underset{\substack{\text{Allyl} \\ \text{thiocarbimide.}}}{C_3H_5.NCS}$$

MYROSIN is the soluble proteid ferment or enzyme of the *cruciferæ*, though its existence is probably not confined to plants of this order.[1] Cruciferous plants abound in highly complex glucosides, which, under the influence of myrosin, split up into glucose and various sulphuretted compounds, among which iso-thiocyanates or thiocarbimides are the most abundant and characteristic. Thus black mustard, white mustard, and mignonette root yield respectively the following thiocarbimides:—

Black mustard; Allyl thiocarbimide, ...	$C_3H_5.NCS$
White mustard; Acrinyl thiocarbimide, . .	$C_7H_7O.NCS$
Mignonette root; Phenyl-ethyl thiocarbimide,	$C_2H_4(C_6H_5).NCS$

Allyl thiocarbimide is also yielded by rape-seed, but (according to Ritthausen) not by turnip-seed. Other plants of the order, such as raddish, cabbage and swede, also yield sulphuretted oils which are not improbably thiocarbimides, though their exact nature is not known.

Myrosin is prepared by extracting ground white mustard seed with cold water, concentrating the filtered liquid to a syrup below 40° C., and then precipitating the myrosin by the minimum quantity of strong alcohol. The precipitate is washed with alcohol until the filtrate is no longer coloured yellow by ammonia, nor reddened by ferric chloride even after addition of water. Thus obtained, myrosin is soluble in cold water to form a transparent, colourless, viscous liquid, which froths on agitation.

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[1] Besides being present in *Sinapis nigra* and *S. alba*, myrosin is contained in the seeds of *Raphanus sativus*, *Brassica napus*, *B. oleracea* and *B. campestris*, *Alliaria officinalis*, *Cheiranthus cheiri*, *Draba verna*, *Cardamine pratensis* and *C. amara*, and *Thlaspi arvense*. The localisation of myrosin in the seed has been the subject of an elaborate research by Guignard (see *Pharm. Jour.*, [3], xxiii. 992).

The aqueous solution of myrosin behaves in the main like one of albumin. It is coagulated by heat and by excess of alcohol, and thereby loses its power of decomposing myronic acid; but is said to recover this by immersion in water for a day or two. Myrosin does not act on amygdalin, the glucoside of bitter almonds. Its action on myronic acid and other glucosides of the *Cruciferæ* is remarkable in not involving either elimination or assimilation of the elements of water. The most favourable temperature for the action of myrosin is a little below 50° C. Above that point it is less powerful, and is coagulated and rendered inactive at about 70°. The fermentation is at once arrested by the addition of 0·3 per cent. of hydrochloric acid.

Myrosin is probably the ferment of all cruciferous seeds, but the glucosides of different species vary considerably in their susceptibility to the action of the ferment. Thus the glucoside of turnips (*Brassica napus*) is very slowly acted on by the ferment co-existing with it in the seeds, which ferment affects the glucoside of *Raphanus sativus* much more energetically and decomposes myronic acid as readily as does the myrosin natural to mustard. (W. J. Smith, *Zeit. physiol. Chem.*, xii. 419; abst. *Jour. Chem. Soc.*, liv. 869; and *Pharm. Jour.*, [3], xviii. 1087.)

It is remarkable that the amount of myrosin contained in black mustard seed is usually insufficient to act on all the glucoside present, whereas the myrosin of white mustard seed is more than sufficient to transform all the sinalbin co-existent with it. From this it follows that a greater yield of the volatile oil of mustard (allyl thiocarbimide) will be obtained from a mixture of black with white mustard than from black mustard only, although white mustard itself yields mere traces of volatile oil (see page 111). Hence for making mustard plasters, &c., the British Pharmacopœia (1885) prescribes the use of a mixture of (ground) black and white mustard seeds, but neglects to state the proportions in which they are to be mixed. The proportion of myrosin contained in white mustard seed is commonly stated at about 19 per cent.; the amount in black mustard is very variable, being said to range from 2 to 18 per cent. These figures must, however, be accepted with much caution.

Guignard has further found (abst. *Pharm. Jour.*, [3], xxv. 541) that myrosin (or, at any rate, a ferment decomposing myronic acid) is not only present in the *Cruciferæ* but exists also in *Limnanthaceæ*, *Tropæolaceæ*, *Residaceæ*, and *Papayaceæ*, and is found in the roots, stems, leaves, and seeds. The wing of *limaria* seed contains it in abundance.

SINALBIN, $C_{30}H_{44}N_2S_2O_{16}$, is the glucoside of white mustard seed, and is probably present to a limited extent also in the seeds of black mustard.

For the isolation of sinalbin, white mustard seed should be finely ground, and thoroughly freed from fixed oil by pressure and treatment with benzol or carbon disulphide. The powder is then exposed to the air till the solvent has evaporated, and then added to four times its weight of boiling methylated spirit. The liquid is boiled under a reflux condenser for half an hour, to complete the coagulation of the myrosin, and then filtered boiling hot. On cooling and standing, sinalbin crystallises out, the accompanying salts of sinapine remaining in solution. The crystals are separated, washed with carbon disulphide, dissolved in a minimum of warm water, the solution decolorised by animal charcoal, filtered, and the sinalbin reprecipitated by excess of strong alcohol. It is then recrystallised from boiling spirit.

Thus prepared, sinalbin forms small pearly needles of a faint yellowish tint. When heated it melts to a yellow liquid, which at a higher temperature decomposes with evolution of fumes of disagreeable odour. Sinalbin is readily soluble in water, and dissolves in about 3 parts of boiling rectified spirit; but is only sparingly soluble in cold absolute alcohol. In ether and carbon disulphide it is insoluble.

Sinalbin solutions are neutral to litmus, and optically inactive. They reduce Fehling's solution with precipitation of cuprous sulphide. Mercuric chloride gives a white crystalline precipitate of a mercury compound of sinapine, glucose being formed. Silver nitrate produces a white precipitate, while the strongly acid filtrate contains sinapine and dextrose. The precipitate is decomposed by sulphuretted hydrogen with formation of sinapine sulphate and acrinyl cyanide. Caustic alkalies, even in traces, colour solid sinalbin intensely yellow, which nitric acid changes to blood-red. Sinalbin gives no precipitate with barium chloride.

The most interesting and characteristic reaction of sinalbin is that with myrosin, or with a cold aqueous extract of white mustard, which rapidly converts it into dextrose, sinapine sulphate, and acrinyl iso-thiocyanate or thiocarbimide, thus :—

$$C_{30}H_{44}N_2S_2O_{16} = C_6H_{12}O_6 + C_{16}H_{23}NO_5,H_2SO_4 + C_7H_7O.NCS.$$

When boiled with caustic soda, sinalbin yields sodium sulphate and thiocyanate.

Sinapine, $C_{16}H_{23}NO_5$, exists as thiocyanate in white (and black) mustard seed, and the sulphate results from the decomposition of sinalbin by the action of myrosin (see above). For its preparation, Remsen and Coale (*Amer. Chem. Jour.*, vi. 50) recommend that 100 lbs. of white mustard seed should be pressed to separate oil, and extracted with alcohol. The filtered and concentrated liquid is mixed with a small quantity of an alcoholic solution of potassium thiocyanate, when crystals of sinapine thiocyanate slowly separate. Remsen and Coale obtained a yield of 80 grammes of the salt, and consider their method superior to that of Babo and Hirschbrum, who have described a similar process (*Annalen*, lxxxiv. 10).

To obtain the free base, an aqueous solution of sinapine thiocyanate should be treated with silver sulphate in known amount, and the liquid filtered from the silver thiocyanate treated with baryta-water in quantity just sufficient to precipitate the sulphate. The filtered liquid is an aqueous solution of sinapine. The base cannot be obtained in a solid state. On evaporating the aqueous solution it is decomposed with great facility into sinapic acid[1] and choline:—

$$C_{16}H_{23}NO_5 + 2H_2O = C_{11}H_{12}O_5 + C_5H_{15}NO_2.$$

The aqueous solution of sinapine has an intense yellow colour. It is strongly alkaline, and precipitates solutions of copper (green), mercury (brown), and silver (brown).

Sinapine forms crystallisable salts with acids. The *sulphate*, $B,H_2SO_4 + 2$ aqua, forms rectangular plates, or slender monoclinic prisms, easily soluble in water and boiling alcohol to form solutions of an acid reaction. It may be obtained by adding sulphuric acid to an alcoholic solution of the thiocyanate. The *nitrate* and *hydrochloride* crystallise in needles very readily soluble in water. On adding mercuric chloride to the solution of the latter salt, $B,HHgCl_3$ is precipitated in brilliant needles, sparingly soluble in cold water, but readily soluble in boiling water.

Sinapine Thiocyanate, $C_{16}H_{23}NO_5,HCNS$, exists ready-formed in white mustard seeds. It crystallises in colourless needles, which are yellow if impure, but become colourless in presence of a trace of free acid. The salt melts at 176°, and is only sparingly soluble in cold water or alcohol, but dissolves readily on heating. The solutions give a red coloration with ferric chloride.

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[1] *Sinapic acid* has probably the constitution of butylene-gallic acid, $C_4H_8:O_2:C_6H_2(OH).COOH$. It is best prepared by boiling sinapine thiocyanate with baryta-water, filtering, and decomposing the washed precipitate of barium sinapate with dilute sulphuric or hydrochloric acid. When recrystallised from alcohol and water, the sinapic acid melts at 186° to 192°. Its salts have not been obtained crystallised.

Sinalbin Mustard Oil, $C_6H_4(OH).CH_2.NCS$, has the constitution of acrinyl or para-hydroxybenzyl iso-thiocyanate (thiocarbimide). It is produced, together with sinapine sulphate and dextrose, by the action of myrosin on sinalbin (page 103). It may be obtained by removing the fixed oil from ground white mustard seed by benzol or carbon disulphide, and treating the residue with cold water. The glucose, sinapine sulphate, and myrosin dissolve, while the sinalbin mustard oil remains with the insoluble matter, from which it may be extracted by treatment with ether. Acrinyl thiocarbimide is a yellow oily liquid, having a very pungent, burning taste. It readily produces blisters on the skin, but is not so powerful a vesicant as allyl thiocarbimide, the volatile oil of black mustard. The odour of mustard is not apparent till the oil is warmed, and the compound is but slightly volatile even in a current of steam. It is insoluble in water, but dissolves readily in alcohol and in ether. It also dissolves in caustic alkalies, after which treatment, but not previously, the acidulated solution gives a red coloration with ferric chloride. Sinalbin mustard oil has been obtained synthetically by H. Salkowski (*Ber.*, xxii. 2384), by treating para-hydroxybenzyl-amine with carbon disulphide, and the resultant compound with mercuric chloride. Like the corresponding allyl compound, acrinyl thiocarbimide readily loses sulphur with formation of the nitril of para-hydroxyphenyl-acetic acid, a body melting at 69°, soluble in alcohol, ether, or warm water, and decomposed by boiling caustic soda with evolution of ammonia and formation of ortho-hydroxy-phenyl-acetic acid, $C_6H_4(OH).CH_2.COOH$. This is a substance crystallising in colourless prisms, melting at 144·5°, and soluble in alcohol, ether, and hot water. Its barium and calcium salts form crystals only sparingly soluble in cold water (see Will and Laubenheimer, 1880, *Annalen*, cxcix. 150; abst. *Jour. Chem. Soc.*, xxxviii. 265; also H. Salkowski, *Ber.*, xxii. 2137; abst. *Jour. Chem. Soc.*, lvi. 1173).

MYRONIC ACID or SINIGRIN, $C_{10}H_{19}NS_2O_{10}$, is the glucoside of black mustard seeds. It is best prepared by finely powdering black mustard seed, expressing as much of the fixed oil as possible, and extracting the remainder by treatment with benzol.[1] The residue is re-powdered, and added gradually to 3 or 4 parts of boiling methylated spirit to render the myrosin insoluble. The liquid is boiled for half an hour, and then evaporated to dryness at 100°. The residue is re-powdered and extracted cold water, which dissolves out the potassium myronate. filtered liquid is evaporated with addition of a little barium bonate, the residue extracted with boiling methylated spirit, the liquid filtered while hot. On standing for some days, p sium myronate crystallises out in long silky needles, which be purified by recrystallisation from boiling alcohol.

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[1] The preliminary removal of the fixed oil by pressure and treatment with benzol is not essential.

Free myronic acid may be obtained by mixing saturated aqu solutions of 100 parts of potassium myronate and of 38 par tartaric acid, and adding 4 or 5 measures of alcohol. The potassium tartrate which crystallises out is removed by filtra and the filtrate evaporated to a syrup.

Thus obtained, myronic acid forms an odourless, bitter, syrup. It has a strong acid reaction, and is readily solubl water and alcohol, but is insoluble in ether.

Myronic acid is very unstable. It is readily decomposed heated, and even the dilute aqueous solution evolves sulphur hydrogen when boiled for some time. Boiling baryta-wate composes it with precipitation of barium sulphate and form of allyl iso-thiocyanate. Caustic soda and potash gives ally phide, thiocyanate and cyanide, with ammonia and glucose. centrated hydrochloric acid sets free sulphuric acid. When my acid is treated with zinc and dilute hydrochloric acid, hal sulphur is evolved as sulphuretted hydrogen, the solution containing glucose, sulphuric acid, and ammonia. Dilute act similarly but more gradually.

The most interesting reaction of myronic acid is that myrosin, the soluble albuminous ferment of mustard seed. treated in aqueous solution with this substance, an odo mustard oil is developed in a few minutes, and after some complete decomposition occurs with formation of glucose, phuric acid, and allyl thiocarbimide or iso-thiocy (page 107). The same reaction occurs on mixing ground mustard seeds with water, according to the following equatic

$$C_{10}H_{18}KNS_2O_{10} = C_6H_{12}O_6 + KHSO_4 + C_3H_5.NCS.$$[1]

Myronic acid forms a series of colourless, odourless salts, all of which are soluble, while those of some of the light are crystallisable.

Potassium Myronate, $KC_{10}H_{19}NS_2O_{10}$, exists in black m seed, and may be isolated therefrom by the process already described. The yield is only about 0·5 per cent. The salt crystallises from alcohol in concentrically arranged groups of silky needles, and from water in short, glassy, anhydrous rhombic prisms. Potassium myronate has a cooling bitter taste, a neutral reaction, and dissolves very readily in water, sparingly in weak spirit, and is nearly insoluble in absolute alcohol. It is not dissolved by ether, chloroform, or benzene.

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[1] J. Attfield (*Manual of Chemistry, General, Medical, and P ceutical*) gives $K_2C_{20}H_{38}N_2S_4O_{19}$ as the formula of potassium myronat represents potassium hydrogen sulphite (not sulphate) as being formed reaction.

The reactions of potassium myronate are the same as those of myronic acid. When an aqueous solution of the salt is mixed with a cold aqueous extract of white mustard, the liquid rapidly becomes turbid, acquires an acid reaction, and evolves an odour of mustard oil. The solution then contains dextrose and a sulphate. The turbidity is due to separation of sulphur and of an organism resembling yeast. A similar decomposition of potassium myronate is not induced by treatment with emulsin or an aqueous extract of bitter almonds, by saliva, nor by yeast.

With neutral lead acetate, potassium myronate gives a yellowish-white precipitate, soluble in acetic acid. Mercurous nitrate gives a similar precipitate, which is decomposed by heat with formation of mustard oil.

On treating a solution of potassium myronate with silver nitrate, nitric acid is set free and a white curdy precipitate formed, the reaction being apparently represented by the equation:—

$$C_{10}H_{18}KNS_2O_{10} + 2AgNO_3 = C_4H_5Ag_2NS_2O_4 + C_6H_{12}O_6 + KNO_3 + HNO_3.$$

The silver compound, when heated either alone or with water, yields silver sulphate and mustard oil, silver sulphide and crotonitril being also formed. If decomposed by sulphuretted hydrogen, the silver compound also yields the two latter products, together with sulphuric acid and free sulphur:—

$$C_4H_5Ag_2NS_2O_4 + H_2S = C_4H_5N + Ag_2S + H_2SO_4 + S.$$

Myronic acid is absent from the seeds of white mustard, but is present in rape seed (*Brassica rapa*). According to Ritthausen (*Jour. prakt. Chem.*, [2], xxiv. 273), it does not exist in turnip seed (*B. napus*), but apparently some analogous compound is present which yields a volatile sulphur compound distinct from oil of mustard when the ground seeds are treated with water.

VOLATILE OIL OF MUSTARD has the constitution of allyl isothiocyanate or allyl thiocarbimide:—$C_3H_5.NCS$.[1] As already explained, it does not exist ready-formed in the mustard seed, but is a product of the action of the ferment myrosin on myronic acid, a glucoside existing in black mustard seed, and in smaller quantities in the seeds of some other cruciferous plants. Myronic acid does not exist, at least in sensible quantity, in the seed of white mustard, and hence no appreciable quantity of allyl thiocarbimide is obtainable from the latter source. In place of it white mustard yields acrinyl thiocarbimide (page 105), which is acrid and pungent to the taste, but practically non-volatile.

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[1] When allyl iodide is heated with the thiocyanate of potassium or silver, allyl thiocyanate is formed. This body is a colourless, oily liquid,

Allyl iso-thiocyanate is a colourless liquid, having a specific gravity of 1·018 at 15°, or of 1·036 at 0° C. It boils at 148°–150° C., and distils readily in a current of steam. Allyl thiocarbimide smells powerfully of mustard, and the vapour excites tears. It has a burning, mustard-like taste, and rapidly blisters the skin. It is slightly soluble in water, and readily soluble in alcohol, ether, and carbon disulphide, the solutions being neutral in reaction and optically inactive. On exposure to light, mustard oil gradually becomes yellow, and gives a deep yellow deposit which contains sulphur and nitrogen, and closely resembles "pseudo-sulpho-cyanogen."

When oil of mustard is boiled for some time with water in a flask furnished with a condensing arrangement, a light, ethereal-smelling oil passes over, which has been identified by Will as crotonitril (allyl cyanide), $C_3H_5.CN$, and is evidently formed from the mustard oil by the loss of sulphur. This decomposition-product is said to occur in commercial oil of mustard in proportions which may reach 50 per cent.

When mustard oil is treated with excess of ammonia, thiosinamine or allyl-thio-carbamide, $CS.N_2H_3(C_3H_5)$ is formed.[1] This reaction is utilised for the determination of mustard oil.

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having an odour at once suggesting that of garlic and of hydrocyanic acid. When heated, it commences to boil at 161°, but the temperature soon falls, and an intense odour of mustard oil is observed, and if the boiling be continued till the temperature falls to 148°–149°, the thiocyanate is found to have suffered complete conversion into the isomeric iso-thiocyanate, or mustard oil:— $C_3H_5.S.CN = C_3H_5.N.SC$. This molecular transposition takes place gradually at the ordinary temperature, and is the more remarkable from the fact that the other ethereal salts of thiocyanic acid are very stable compounds showing no disposition to undergo similar molecular change.

[1] THIOSINAMINE is best prepared by treating mustard oil with three to four times its measure of strong ammonia, and then passing ammonia gas through the liquid till saturated. The new body is deposited in glistening crystals. Thiosinamine is an odourless substance which melts at 74° C. to an oily liquid, which solidifies some time after cooling. It dissolves somewhat sparingly in cold water, but readily on heating, and is deposited again

When treated with recently precipitated lead hydroxide, thiosinamine is converted into allyl cyanamide or sinamine, $CN.NH(C_3H_5)$, a substance of extremely bitter taste and strong alkaline reaction, which decomposes ammoniacal salts, precipitates many metallic solutions, and combines with mercuric and platinic chlorides.

Mustard oil mixes with strong sulphuric acid without great coloration, but decomposition subsequently ensues. Heated in alcoholic solution with zinc and hydrochloric acid, mustard oil evolves sulphuretted hydrogen and methane, and forms allylamine, $C_3H_5.H_2N$. Boiled with caustic soda and a little lead acetate, oil of mustard yields black lead sulphide. When heated with strong nitric acid or bromine, oil of mustard yields a number of products, among which sulphuric acid may be recognised by diluting largely and adding barium chloride. Several methods of determining small quantities of mustard oil have been based on the formation of sulphate by treatment with oxidising agents.

Oil of mustard is occasionally prepared artificially by distilling allyl sulphate or allyl iodide, C_3H_5I, with potassium thiocyanate. A sample, probably prepared in the former manner, was found by E. Mylius to contain:—Allyl iso-thiocyanate, 92·2 per cent.; carbon disulphide, 0·8 per cent.; hydrocyanic acid, ·02 per cent.; polysulphides (chiefly allyl trisulphide), 4·0 per cent.; and non-volatile bodies containing both nitrogen and sulphur, 3·0 per cent. According to C. Schacht, this must have been an exceptionally impure sample.

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on cooling in tufts of monoclinic needles. In alcohol and ether, thiosinamine is readily soluble. Allyl-thio-carbamide or allyl-thio-urea has the characters of a weak base, and resembles urea and other carbamides in combining with salts. Taken in moderate doses it occasions palpitation and sleeplessness, while ammonium thiocyanate is found in the urine.

Thiosinamine is oxidised by nitric acid, but boiling with the concentrated reagent even for thirty minutes does not suffice to convert the whole of the sulphur into the form of sulphate. On adding silver nitrate to an aqueous solution of thiosinamine, a white curdy precipitate is formed, soluble in excess of the base but permanent in presence of excess of the silver salt. The precipitate is readily soluble in alcohol. Mercuric chloride reacts similarly with thiosinamine. Platinic chloride produces in solutions of thiosinamine a curdy, orange-yellow precipitate, insoluble in cold water or in excess of thiosinamine. In hot water it melts, rising to the surface before dissolving, and on cooling separates as a sticky mass, which is readily soluble in alcohol. Mayer's reagent gives with thiosinamine a dirty white precipitate, which, in a few hours at the ordinary temperature or immediately on heating, forms oily drops. Nessler's solution yields an insoluble yellow precipitate. Picric acid does not precipitate thiosinamine, except from strong solutions.

The purity of commercial oil of mustard may be tested in the following manner:—If two or three drops of the sample be allowed to fall on cold water, they should sink to the bottom on very slight agitation and should remain perfectly clear. A slight admixture with petroleum-spirit causes the drops to remain at the surface. If the oil contain 5 per cent. of ethylic alcohol or amylic alcohol, the drops will become opalescent. One c.c. of the oil, when heated in a shallow dish to 40°–50° C. for two hours, should volatilise without residue; thus proving the absence of fatty oils, phenol, nitrobenzene, oil of cloves, &c.

According to Hager (*Year-Book Pharm.*, 1880, page 85), a solution of 10 drops of mustard oil in 4 c.c. of pure absolute alcohol, when mixed with 2–3 c.c. of strong solution of ammonio-sulphate of copper (prepared by adding ammonia to a saturated aqueous solution of copper sulphate until the precipitate is re-dissolved), produces an ultramarine-blue precipitate, which should not change its colour. In the presence of a trace of carbon bisulphide, the precipitate first turns violet-brown, and then gradually changes to reddish-brown. The presence of amyl alcohol, phenol, or oil of cloves would also cause a change of colour. According to the same authority, carbolic acid is best detected in mustard oil by shaking the sample with water for some time, allowing it to stand for fifteen minutes, filtering, and testing the filtrate with a few drops of solution of ferric chloride, which, in the presence of the adulterant, will produce a blue coloration. Or the sample may be diluted with five times its measure of alcohol, and a drop of tincture of ferric chloride added, when, in presence of phenols, a blue or violet colour will be produced.

The United States Pharmacopœia (1890) gives the following additional tests for commercial oil of mustard:—

If 3 grammes of the oil be gradually treated with 6 grammes of sulphuric acid, the liquid being kept cool, the mixture on subsequent agitation will evolve sulphur dioxide, but will remain of a light yellow colour, and at first practically clear, becoming afterwards thick and occasionally crystalline, while the pungent odour of the oil will disappear.

The oil should distil completely between 148° and 150° C., and both the first and last fractions of the distillate should have the same specific gravity as the original oil (absence of crotonitril, alcohol, chloroform, carbon disulphide, petroleum, or fatty oils).

If a mixture of 3 grammes of the oil with an equal weight of alcohol be shaken in a small flask with 6 grammes of ammonia (sp. gr., 0·960) it will become clear on standing for some hours, or rapidly if warmed to 50° C., and will usually deposit, without becoming coloured, crystals of thiosinamine (page 108). To determine the amount of this body obtainable from the sample, the mother-liquor should be decanted from the crystals and gradually evaporated in a flat dish on the water-bath, adding fresh portions of the liquid only after the ammoniacal odour of each preceding portion has disappeared. The crystals are then rinsed out of the flask with a little alcohol and added to the concentrated mother-liquor, which is then evaporated to dryness on the water-bath and dried till constant. The residue of thiosinamine forms on cooling a brownish crystalline mass, melting at 70° C., and having a leek-like but not a pungent odour. The yield from 3 grammes of mustard oil should not be less than 3·25 (=108·3 per cent.) nor more than 3·50 (=116·7) grammes.[1]

F. A. Flückiger (*Jour. Soc. Chem. Ind.*, viii. 472) has also shown that the ferric thiocyanate reaction cannot be employed for the purpose of detecting carbon disulphide in mustard oil, since pure mustard oil, warmed with a little alcohol and ammonia, and the excess of ammonia removed by heating in the water-bath, gives ammonium thiocyanate, which would, of course, give the red coloration with ferric chloride.

Cruciferous seeds all yield traces of allyl thiocarbimide or closely allied compounds on treatment with water, but the quantity is very minute, except in the case of black mustard seeds. Thus V. Dircks (abst. *Jour. Chem. Soc.*, 1883, 245) obtained from black mustard-seed cake 1·39 per cent. of volatile oil; from yellow mustard cake, 0·018; from rape-seed cake, 0·020–0·109 (the proportion of oil apparently decreasing with the age of the cake); from rape seeds, 0·018–0·037; from turnip seeds, 0·038; and from the seeds of *Sinapis arvensis*, 0·006 per cent. of volatile oil. Mustard-seed cake is highly irritating to cattle, and mustard should be rigidly excluded from cattle foods. Hence the determination of the mustard oil in seed cakes is sometimes of considerable practical importance.

For the determination of the pungent volatile oil in cruciferous seeds and seed cakes several methods have been proposed, depending on the distillation of the separation of the volatile oil from

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[1] F. A. Flückiger, to whom this test is due, obtained from pure mustard oil 111, 112, 112·3, and 115·7 per cent. of thiosinamine, against a theoretical yield of 117·7 per cent. He attributes the deficiency to an unavoidable formation of thiocyanate, but this product is reduced to a minimum when a moderate heat is used and the least possible quantity of ammonia is employed.

fixed matters by distillation in a current of steam, and its
quent conversion into some definite and readily-weighed s
compound. The plan usually adopted is to mix the crushe
or oil-cake with about ten times its weight of cold or s
warm water, allowing it to stand for a time varying from
hour to six hours (compare footnote on page 116), and then
ising the oil formed by blowing a stream of open steam t
the flask. V. Dircks (*Landw. Versuchs. Stat.*, xxviii.
abst. *Jour. Chem. Soc.*, 1883, 245), however, insists
importance of adherence to certain conditions in order to
satisfactory results. He recommends that the finely-po
substance should be mixed with ten parts of water, an
mixture allowed to stand for nine hours at 50° C., this
necessary to allow of the easy distillation of the oil.
and air are then blown simultaneously through the mi
and the distillate collected in an alkaline solution of pot
permanganate, which is subsequently treated with
chloric acid and the sulphate formed precipitated as
sulphate.

A. Schlicht (*Zeits. anal. Chem.*, xxx. 661; abst. *Jour.*
Soc., lxii. ii. 1035) recommends the following modified
as simple and trustworthy:—

To the aqueous distillate containing the mustard oil are
20 parts of potassium permanganate and 5 parts of
potash or caustic soda (which reagents must be free
sulphates) for each part of mustard oil supposed to be p
The mixture is shaken for some time in a closed flask, and
heated nearly to boiling. The whole of the sulphur is
oxidised to sulphuric acid. After cooling the solution some
5 c.c. of alcohol should be added for every gramme of pe
ganate previously used. By these means the whole of the
ganese is precipitated. The mixture is then completely
largely diluted, made up to a known volume, and fil
A measured portion of the filtrate is slightly acidified
hydrochloric acid, and treated with a solution of iodi
potassium iodide until a feeble yellow colour remains
after warming. This reproduces any sulphuric acid which
have been reduced by the aldehyde, and also removes the
hyde itself.[1] The sulphuric acid is now determined by pre
tion with barium chloride, and the weight of barium su
multiplied by 0·42492. The product gives the amount of m

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[1] The reduction of sulphuric acid in dilute alkaline solution by al
is highly improbable. Addition of bromine-water would do instanta
and certainly what Schlicht effects by iodised potassium iodide.

oil. Test analyses gave results varying from 99·74 to 99·95 per cent.

G. Ulex (*Zeits. anal. Chem.*, 1883; abst. *Chem. News*, xlvii. 249) shakes up the distillate with bromine (free from sulphuric acid), expels the excess of bromine by heat, and decolorises the liquid with ammonia. This liquid he acidifies with hydrochloric acid, filters, and precipitates with barium chloride. 233 parts barium sulphate precipitated represent 99 parts of mustard oil.

The following method of determining volatile mustard oil in oil cake has been devised by O. Förster (*Jour. Chem. Soc.*, liv. 1350):—25 grammes weight of the powdered substance is made into a thin paste with water, allowed to remain for half an hour,[1] and then heated by the introduction of steam, whereby the oil is volatilised. It is passed into a condenser connected with a 250 c.c. flask, containing 50 c.c. of alcohol saturated with ammonia, the end of the condenser dipping into the liquid. When a volume of about 150 c.c. has distilled, the liquid is allowed to remain for twelve hours in a closed vessel, to ensure the complete conversion of the allyl thiocarbimide into thiosinamine, and afterwards brought to boiling in a beaker, and freshly prepared mercuric oxide[2] is added in quantity sufficient to combine with all the sulphur present. The mixture is then again boiled, and before it is quite cold potassium cyanide is added to remove all excess of mercuric oxide and oxydimercurammonium hydroxide. The mercuric sulphide is then collected on a tared filter, dried, and weighed, and the weight, multiplied by 0·4266, gives the amount of mustard oil.

A small loss may occur, since potassium myronate in presence of myrosin and water yields, besides mustard oil, small quantities of crotonitrile and free sulphur, which remain in the retort. The extent to which this may occur can be estimated by preliminary experiments. Other volatile compounds may be formed, but with alcoholic ammonia and mercuric oxide they also yield mercuric sulphide.[3]

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[1] F. Sutton considers that at least an hour should be allowed.

[2] The mercuric oxide employed is prepared by decomposing 25 c.c. of a 4 per cent. solution of mercuric chloride with caustic potash and boiling the mixture.

[3] Thus diallyl carbimide and diallyl thiocarbimide may be formed, but the sulphuretted hydrogen simultaneously produced reacts with the mustard oil to form carbon disulphide and allylamine or diallyl thiocarbimide; but the carbon disulphide reacts with the alcoholic ammonia to form ammonium thiocarbonate, which by treatment with mercuric oxide yields mercuric sulphide.

COMMERCIAL MUSTARD.

The mustard of commerce should consist of the ground seed of white or black mustard (*Sinapis nigra* and *S. alba*), or of a mixture of the two, which, for reasons given on page 102, is preferable to either kind separately.[1] In India, Central Asia, and Southern Russia the seeds of *Sinapis juncea* are used. True mustard seed has been replaced by the ground seeds of charlock or wild mustard (*Brassica campestris*), and by rape seed (*Brassica napus*).

The earliest tolerably complete analyses of mustard are those published by A. H. Hassall (*Food, Water, and Air*, Feb. 1874), who gave the following as the composition of typical samples of brown and white mustard :—

	Brown Mustard.		White Mustard.	
Moisture, ...	4·84	per cent.	5·35	per cent.
Fixed oil, ...	35·70	,,	35·78	,,
Myronic acid, ...	4·84	,,	none	,,
Sinapine thiocyanate, . .	3·59	,,	10·98	,,
Myrosin and albumin, .	29·54	,,	27·48	,,
Cellulose (by difference), .	16·76	,,	16·29	,,
Ash, ...	4·73	,,	4·11	,,
	100·00		100·00	

In these analyses the cellulose was estimated "by difference," and is probably much above the true amount. The myronic acid was calculated from the amount of volatile oil (allyl thiocarbimide) obtained on distilling the sample with water. The sinapine thiocyanate was deduced from the amount of sulphur, after allowing for that present in the forms of myronic acid and myrosin, the former of which contains 17·24 and the latter about 1 per cent. of sulphur. The myrosin and albumin were estimated by multiplying the nitrogen by 6·25. Working on much the same lines, but employing an improved process for the determination of the volatile oil, Piesse and Stansell in 1880 (*Analyst*, v. 161) found mustard to have the following composition :—

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[1] Both black and white mustard are cultivated in this country, though a considerable quantity is imported. White mustard seeds have a yellowish tint, while black mustard seeds are of a brownish-purple colour. The seeds of white mustard are much larger than those of black mustard. Thus, Piesse and Stansell found that 170 Yorkshire white seeds or 172 Cambridge white seeds weighed 1 gramme, while the same weight of Cambridge brown mustard contained 944 seeds.

Constituents, &c.	WHITE MUSTARD.					BROWN MUSTARD.			
	WHOLE SEEDS.		FARINA.			WHOLE SEEDS.	FARINA.		
	Yorks.	Cambridge.	Superfine.	Fine.	Seconds	Cambridge.	Superfine.	Fine.	Seconds
oisture,	9·32	8·00	...	5·78	6·06	8·52	4·35	4·52	5 63
ixed oil,	25·56	27·51	37·18	35·74	32·55	25·54	36·96	38·02	36·19
llulose,	10·52	8·87	3·90	4·15	9·34	9·01	3·09	2·06	3·26
lphur,	0·99	0·93	1·33	1·22	1·26	1·28	1·50	1·48	1·30
itrogen,	4·54	4·49	5·05	4·89	4·25	4 38	4·94	5·01	4·31
otal proteids,	28·37	28·06	31·56	30·56	26·56	26·50	29·81	30·25	26·06
oluble albumin and myrosin,	5·24	4·58	7·32	6·67	6·11	5·24	6·46	6·78	6·14
queous extract,	27·38	26·29	36·31	36·60	33·90	24·22	31·64	32·78	31·41
olatile oil,	·06	·08	·03	·04	·03	·47	1·44	1·50	1·38
Potassium myronate,	...	...	...	...	...	1·69	5·14	5·37	4·94
otal ash,[1]	4·57	4·70	4·22	4·31	4·30	4·98	5·04	4·84	4·91
luble ash,	0·55	0·75	0·44	0·55	0·33	1·11	1·01	0·98	0·77

The volatile oil which appears in the foregoing analyses is not that existing ready-formed in the seeds, but represents the amount which can be obtained by the hydrolysis of the myronic acid present under the influence of the ferment myrosin. It was determined by Piesse and Stansell in the following manner.

25 grammes of crushed brown mustard seeds are mixed with about a quarter of their weight of white seeds, 300 c.c. of cold water added, and the mixture allowed to stand in a 700 c.c. flask at the ordinary temperature for five to six hours. The flask is then connected with a condenser, and the distillate collected in a small flask containing 30 c.c. of strong ammonia (sp. gr. 0·880). The liquid is distilled as long as drops of oil come over, and the steam no longer possesses the pungent odour of mustard oil. This is generally the case when about 50 c.c. measure has been distilled. The condenser is then well rinsed out with cold distilled water into the receiver (this is necessary), and the latter corked and set aside, with occasional agitation, for twenty-four

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[1] Piesse and Stansell have given the analysis of the ash of mustard. The white and black varieties yield very similar results, the ash consisting mainly of potassium, calcium, and magnesium phosphates, with very little chlorides and no carbonates (*Analyst*, v. 164).

hours, or until the drops of oil have entirely disappeared. The distillate is then evaporated to dryness, and the residue of thiosinamine dried in the water-oven, and weighed. The weight obtained, multiplied by 0·853, gives that of the corresponding *mustard oil*, or if multiplied by 3·578, the weight of *potassium myronate*, from which it was derived, will be obtained. Potassium myronate contains 3·37 per cent. of nitrogen, and yields 23·85 per cent. of volatile oil of mustard.[1]

Piesse and Stansell consider that their results show that in the process of manufacture the sifting chiefly removes the husk and dries the farina, the remaining constituents being correspondingly concentrated. Thus the fixed oil averages 25 per cent. in the seeds, but rises to 37 per cent. in the farina, while the moisture is reduced to one-half and the cellulose to one-third. It is difficult to account for the great increase in the volatile oil, which is three times as great in the brown mustard farina as in the seeds. The broad distinction between the white and brown mustard is, of course, the much greater proportion of potassium myronate in the latter, and hence its greater yield of volatile oil. Qualitatively, brown mustard may be distinguished from white by the characters of the cold water extract. In the case of white mustard, the infusion yields a deep blood-red coloration with ferric chloride, while the reaction is very slight or imperceptible in the case of brown mustard. An aqueous extract of white mustard acquires a marked odour of sulphuretted hydrogen on standing for a few hours; but the extract of black mustard only smells of the volatile mustard oil. Piesse and Stansell's analyses do not show the large portion of myrosin reputed to exist in white mustard as compared with that in the black variety.

F. Sutton, who has had a large experience of Piesse and Stansell's process, states that it gives very fair determinations of the proportion of brown mustard present in a mixture of the two kinds. The calculation is, of course, based on the assumption that the amount of potassium myronate is fairly constant. Taking it at 5·15 per cent., it would yield 1·33 per cent. of thiosinamine, and the weight of the latter compound found, multiplied by 74·2, will be amount of brown mustard in the quantity of the sample employed.

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[1] Piesse and Stansell found that not less than three or more than six hours should be allowed to elapse before commencing the distillation. The yield of volatile oil was sensibly less if this latter limit of time was exceeded, and in forty-eight hours the yield was only two-thirds of the total. F. Sutton considers that five hours is the best time to allow for the digestion.

The following method for the analysis of mustard was published by Leeds and Everhart in 1882 (*Zeits. Anal. Chem.*, xxi.; abst. *Chem. News*, xlvii. 58). The moisture and ash are determined as usual. For the estimation of the oil, a weighed quantity of mustard, previously dried at 105° C., is exhausted with ether in an extraction-apparatus. The ether is then distilled off, and the oil weighed after being dried at 100° C. The oil-free mustard is heated to 100° C. to drive off any remaining ether, and then extracted with proof-spirit. This dissolves the sinapine thiocyanate and potassium myronate, but coagulates the myrosin, and leaves it and the cellulose undissolved, together with any admixture of starch. When the extraction of soluble matter is complete, the spirituous liquid is evaporated in a weighed platinum dish, the residue dried at 105° C., and weighed. It is then ignited, and again weighed. The weight of the potassium sulphate thus obtained, multiplied by the factor 4·782, gives the weight of potassium myronate,[1] and the difference between this and the weight of the residue dried at 105° C. is the sinapine thiocyanate. The residue in the extraction apparatus is freed from alcohol, and treated with a 0·5 per cent. solution of caustic soda. The residue, which consists of crude cellulose (plus any starch which may be present), is dried and weighed. It is then ignited, and the weight of the ash deducted to obtain the weight of the pure cellulose (and starch). The solution containing the myrosin is approximately neutralised by hydrochloric acid, and 50 c.c. of Ritthausen's copper sulphate solution added. The liquid is then exactly neutralised by dilute caustic soda, and the heavy green precipitate of the copper-myrosin compound collected on a weighed filter, dried at 100° C., and weighed. It is then incinerated and the ash deducted, the difference giving the weight of the myrosin. If starch be present as an adulterant the residue remaining after the extraction with proof-spirit may be treated with diastase or dilute acid to convert the starch into glucose, which is then estimated in the usual manner.

An analysis of white mustard, conducted in the foregoing manner, gave Leeds and Everhart the following results as the mean of three concordant analyses. The figures in the last column are deduced from the nitrogen and sulphur by Hassall's method.

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[1] Clifford Richardson (*U. S. Bulletin*, xiii. 1887) remarks that in presence of wheat-flour the dilute alcohol would dissolve sufficient albumin and mineral matters to invalidate the myronate determination. This error would be avoided largely by estimating the sulphate in the ignited residue instead of taking the entire weight as potassium sulphate.

	Found.		Calculated.	
Moisture,	6·83	per cent.	...	per cent.
Fixed oil,	29·21	,,	...	,,
Myrosin and albumin,	28·48	,,	28·52	,,
Potassium myronate,	0·65	,,	0·61	,,
Sinapine thiocyanate,	11·12	,,	10·71	,,
Cellulose (by difference),	19·95	,,	...	,,
Ash,	3·76	,,	...	,,
	100·00	,,		

Nitrogen,	5·34 per cent.
Sulphur,	1·49 ,,

The following is the general composition of ground mustard seeds according to various analyses collected and made by Clifford Richardson (*U. S. Bulletin*, xiii. 182):—

	From Entire Seed.	From Mustard Cake.
Water,	3 to 7 per cent.	3 to 7 per cent.
Ash,	4 ,, 6 ,,	4 ,, 6 ,,
Volatile oil,[1]	5 ,, 2 ,,	5 ,, 2 ,,
Fixed oil,	31 ,, 37 ,,	16 ,, 18 ,,
Crude fibre,	5 ,, 18 ,,	5 ,, 18 ,,
Albuminoids,	25 ,, 32 ,,	25 ,, 32 ,,

Adulterations of Mustard.[2]

Mustard was formerly extensively sophisticated,[3] but the enforcement of the Adulteration Acts has created a great improvement in this respect.[4]

Terra alba (calcium sulphate) was at one time used to a considerable extent as an adulterant of mustard[5]; *sand* has been found in America, and *chalk* is also said to have been employed. A determination of the ash left on igniting the sample would suffice for the detection of these and other mineral adulterants, since pure mustard flour never yields much over 5 per cent. of ash.[1] A low ash, on the other hand, is indicative of the presence of wheat-flour or other cereal admixture.

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[1] This appears to be the volatile oil existing as such in the mustard, and not allyl thiocarbimide purposely formed and determined.

[2] The methods of detecting adulterations of mustard were fully described by the author, in 1874 (*Chem. News*, xxx. 116), but, apparently owing to its omission from the index to the volume in which it appeared, the article has escaped the attention of subsequent compilers.

[3] In an instance communicated to the author (which, if not true, is at least *ben trovato*), a firm who were in financial difficulties and unable to obtain mustard seed on credit, carried on their business for many months by manufacturing "mustard" from a mixture of rape seed, wheat-flour, turmeric, and cayenne pepper.

[4] On November 15, 1895, a shopkeeper in the south of London was convicted of selling mustard containing 70 per cent. of flour and turmeric.

[5] It is asserted that plaster of paris was in one case used so liberally as to cause the mustard to "set" when made into a paste with water.

Chrome-yellow is said to have been employed for colouring mustard, but the substance now used in England for this purpose is *turmeric*. This colouring matter is not commonly used in quantity sufficient to add perceptibly to the weight or bulk of the mustard, but, as the object of its employment is simply to cover the paleness of tint caused by the addition of wheat-flour or other diluent, its detection is occasionally of interest. This can be effected by the microscope, the cells of turmeric having a bright yellow colour and a characteristic appearance. They contain starch, and hence are turned blue by iodine. Turmeric may also be conveniently detected by the boric acid reaction described in Part i. page 359. A small quantity (about 1 gramme) of the sample of mustard is boiled with methylated spirit, and the filtered liquid concentrated and treated with boric acid, &c.

Martius' Yellow, the calcium salt of dinitro-alphanaphthol (Part i. page 154), $[C_{10}H_5(NO_2)_2O]_2Ca+6Aqua$, was found by Waller and Martin in four out of fourteen samples of mustard purchased in New York city in 1884 (*Analyst*, ix. 166). It can be detected by treating the sample with cold alcohol, agitating well for a few minutes, and filtering. The filtrate is evaporated to dryness, the residue taken up with water, and white wool immersed in the filtered liquid, which, in presence of naphthol-yellow or analogous coal-tar colouring matter, will be dyed a bright yellow. If the dyed fragment of wool be wrapped in white paper and heated to 120° in an air-bath, part of the yellow colour will be transferred to the paper. Hot water or dilute ammonia dissolves out the colouring matter, and the yellow solution is decolorised by hydrochloric acid, a yellow precipitate being produced (distinction from picric acid).

F. Sutton has found Martius' yellow in mustards of French, German, and American manufacture; and in some American samples a coloured earth known as *Dutch yellow*, which can be readily detected in the ash.

Cayenne Pepper or Capsicum was formerly often used to impart pungency to diluted mustard. It is recognisable under the microscope by its characteristic structure, and can also be readily detected in the following manner:—About 1 gramme of the sample of mustard is boiled for a short time with alcohol, the liquid filtered, and evaporated to dryness at 100°. The residue is then *tasted*, when the pungent biting flavour of cayenne will be easily perceived. A still more striking test is to heat the dry alcoholic extract, and smell the fumes, when an overpowering heat in the lungs, irresistibly compelling coughing, is produced if cayenne is present. The fumes from pure mustard are not irritating, but ginger produces a somewhat similar effect.

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[1] Waller and Martin, in 1884, found 13 per cent. of ash (containing calcium sulphate) in mustard purchased in New York city.

Wheat-flour is a very usual addition to mustard, and it has been frequently alleged that its use is *necessary* as a preservative. This contention is disproved by the fact that some manufacturers have never countenanced such an addition, and that nearly all makers now supply genuine unmixed mustard of various qualities and prices, these varying according to the proportions of black and white seeds present.

It appears, however, to be an established fact that the addition of a moderate proportion (*i.e.*, 8 to 10 per cent.) of rice or wheat-flour to the finer qualities of table mustard, especially such as contain a large proportion of black mustard, materially improves the keeping qualities of the article, both in a dry state and when mixed with water. F. Sutton states that if such mustards are shipped to Australia or India in a pure state they invariably turn dark in colour, and become lumpy, so as to be quite useless for table purposes; and that the same effect is produced in a greater or less degree when kept in shops above the average temperature. It is evident that the addition to a moderate extent of such diluents as flour does not necessarily imply fraud, since it is quite possible to manufacture an unmixed mustard consisting mainly of white seed at an exceedingly low price. A mustard largely advertised as pure is of this character, and as a table mustard is of very poor quality. Black mustard seed is not only higher in price than the white seed, but, owing to the small size of the seeds, the yield of farina is so much less than that from white seed that the cost is enhanced out of all proportion. It may be added that the Government victualling yards manufacture all the mustard required for the use of the navy, and mix a certain proportion of rice-flour with the black and white mustards, in order that the article may better stand variations of climate.

The unacknowledged addition of wheat-flour or other diluent to mustard is an offence under the Sale of Food and Drugs Act; but the addition is permitted if duly announced by label. Such mixed articles are commonly known as "mustard condiment."[1]

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[1] Regarded merely as a condiment, there can be no harm in selling diluted mustard, provided that the purchaser is informed of its true nature; but since mustard is also employed as a remedy, the practice of diluting it is

The mere detection of wheat-flour or other foreign farina in mustard presents no difficulty, since mustard contains no starch naturally. The sample should be boiled with water, the supernatant liquid decanted, and when cold treated with a solution of iodine, which should be added gradually, so as to avoid a large excess while ensuring the use of sufficient of the reagent to leave some iodine uncombined. The production of a blue colour proves the presence of added starchy matter, and the intensity of the reaction will afford some indication of the amount present. The nature of the farinaceous matter can of course be ascertained from the microscopic character of the starch, which method of examination will also give an idea of the probable amount present.

The quantitative determination of the amount of farinaceous matter added to mustard presents considerable difficulty, and strictly accurate results are at present unattainable. In 1874, the author proposed to deduce the amount of added farina from the deficiency of fixed oil in the sample. The results of Hassall (page 114) and the writer had shown that various commercial mixtures of white and brown mustards contained an approximately constant proportion of fixed oil, which averaged 35 per cent. As wheat-flour and similar diluents contain only trifling quantities of oil, the proportion of such matters in an adulterated mustard could be ascertained by multiplying the percentage of fixed oil, as determined by extraction with ether or benzol, by 2·857. This indirect method is invalidated if a portion of the oil natural to the mustard has been extracted by pressing the seed, a plan which is said to be practised by some manufacturers. Again, the results

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liable to have serious consequences. If a mustard plaster were required in a case of emergency, or mustard and water were administered as an emetic in a case of poisoning, it is evident that a diluted article would be objectionable, and its substitution for genuine mustard might result in loss of life.

On this point, however, F. Sutton writes to the author:—"The objection urged on medical grounds to mustards containing a moderate quantity of wheaten or rice-flour when used as sinapisms or as an emetic seems scarcely tenable, inasmuch as it is possible to have a perfectly genuine mustard which possesses less than half the vesicating or stimulating power of one which may contain 10 per cent. of wheaten or rice-flour. The article chiefly required in these cases is the black mustard. It is true that white mustard will irritate the skin or the mucous membranes, but its effects are slower and far more disagreeable, and even dangerous in the end, than with the farina of black mustard.

"The mustard leaves or sinapisms now in common use are made by extracting all the fixed oil from a mixture of black and white mustard flour with ether, benzol, or carbon disulphide, and then attaching the dry powder to fine muslin or cambric."

will be falsified if the deficiency of oil due to dilution with flour, &c., has been made good by adding a foreign oil, a practice of the existence of which the results of Waller and Martin afford some evidence. Hence the determination of the fixed oil, while useful as an independent method of corroboration, must be received with some caution as a reliable indication of the extent of adulteration of mustard.[1]

Some chemists have determined the proportion of admixture in mustard by ascertaining the amount of sugar produced by boiling the sample with dilute acid, but the formation of glucose from the natural constituents of the mustard wholly invalidates this method if applied directly to the sample. Nevertheless, the determination of the starch, after the removal of the oil and glucosides, affords one of the few available means of estimating the proportion of farinaceous adulterants in mustard.[2] This is preferably effected by exhausting the mustard with ether and proof-spirit successively. In the residue the starch can be determined by any of the ordinary methods. 72 parts of starch represent 100 of wheat-flour.

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[1] F. Sutton, who has had a large experience in the analysis and manufacture of mustard, in a communication to the author, writes as follows: "The percentage of oil extracted by ether, multiplied by the factor 2·85, gives as near an approximation to the truth as can be obtained in calculating the amount of real mustard flour in any mixture.

"It is true that the fixed oil varies to a considerable extent in the seeds of both kinds of mustard, according to the soil, climate and locality, and to the harvesting of the seed. The proportion of this oil has been found by me to vary from 30 per cent. in the farina of poor black seed to 47 per cent. in seed of very exceptional quality; in white seed, from 23 per cent. in very common kinds to 38 per cent. in the very best. But these extremes are not by any means general; and the mustard manufacturer who values his reputation will assuredly avoid the use of the commoner kinds, as they are bad both in colour and flavour. The use to which these commoner kinds are put is the manufacture of mustard cake for manure, in place of rape cake. Large quantities of mustard cake are used both in England and on the Continent. The price at which the common mustard seed is sold enables the crusher to make a profit both out of the oil, which is sold to oil-refiners for mixing with rape oil, and out of the cake, which is sold to the farmer. The chief manurial constituent is the nitrogen, which ranges from 3 to 3½ per cent.

"It happens, therefore, in actual practice that the usual mixtures of white and black seed yield about 35 per cent. of fixed oil. If an exceptional proportion of black seed is used, the factor will not be available; but this can be checked by the determination of the myronic acid, or less exactly by the microscope.

"The proportion of any diluent of a starchy nature may also be checked by an estimation of the sulphur of the sample, which in genuine mustard farina averages about 1·4 per cent.; but this method is useless where the admixture amounts to no more than 8 to 10 per cent."

[2] F. Sutton considers this method less satisfactory than a determination based on the proportion of fixed oil.

Saponins.

Under the generic name of "saponin" are classed, or confused, a number of closely analogous glucosides, the great majority of which have been proved to be homologous bodies having the general formula $C_nH_{2n-8}O_{10}$.

R. Kobert (*Chem. Centr.*, 1893, i. 32) has compiled a classified list of 140 plants in which bodies of the saponin class have been detected. He gives the following list of such as have the above general formula :—

$C_{17}H_{26}O_{10}$:—Saponin i, Senegin, Quillaja-sapotoxin, Sapindus-sapotoxin, Gypsophila-sapotoxin, Agrostemma-sapotoxin.

$C_{18}H_{28}O_{10}$:—Saponin ii, Schiedeberg's Digitonin, Saporubrin, Senegin, Assamin.

$C_{19}H_{30}O_{10}$:—Saponin iii or Quillain, Quillajic acid, Polygalic acid, Herniaria-saponin.

$C_{20}H_{32}O_{10}$:—Cyclamin, Paschki's Digitoxin, Merck's Quillajic acid, Sarsaparil-saponin, Smilacin.

$C_{22}H_{36}O_{10}$:—Sarsasaponin.

$C_{26}H_{44}O_{10}$:—Parillin.

$C_{29}H_{50}O_{10}$:—Melanthin.

There is some doubt whether certain of the above substances really belong to the $C_nH_{2n-8}O_{10}$ series, while, on the other hand, dulcamarin, $C_{22}H_{34}O_{10}$, and an isomer of syringin, $C_{17}H_{26}O_{10}$ (from the bark of *Syringa vulgaris*), on further investigation will probably be found to do so. Paridin, $C_{16}H_{28}O_7$, and some other glucosides of quite different composition appear from their properties to belong to the saponin class.

From the foregoing statement it appears that the lowest members of the saponin series have the empirical formula $C_{17}H_{26}O_{10}$, which is the same as that of the crystalline body syringin,[1] identified by Körner as hydroxymethyl-coniferin hydrate. The amorphous substance of this composition prepared by Kruskal agrees with syringin in being a glucoside, and giving a characteristic reaction with sulphuric acid, but further comparative examination is still wanting.

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[1] SYRINGIN, or dihydroxymethyl-coniferin, $C_{17}H_{24}O_9 + H_2O$, is a glucoside occurring in the bark of the lilac, *Syringa vulgaris*, and of the privet, *Ligustrum vulgare*. It crystallises in long, slender, white needles, sparingly soluble in cold water, more readily in hot. It becomes anhydrous at 100°, and melts at 191°. Syringin does not form insoluble compounds with metallic salts. It does not reduce Fehling's solution. With mineral acids it reacts similarly to coniferin. By the action of emulsin, syringin is split up into dextrose and syringenin, or dihydroxymethyl-coniferyl alcohol, $(CH_3O)_2C_6H_2(OH).C_3H_4.OH$, a body which resembles coniferyl alcohol (Körner, *Chem. Centr.*, 1888, page 1098 ; abst. *Jour. Chem. Soc.*, lvi. 159).

From Kobert's researches it appears that saponins of the same formula, and giving identical chemical reactions, when tested pharmacologically show an enormous difference in the intensity of their toxic action. The saponin of corn-cockle (*Agrostemma githago*) is absorbed both by the subcutaneous tissue and the larger intestines, and thus acts as a dangerous poison.

The following table, due to J. C. Umney (*Pharm. Jour.*, [3], xxi. 887), shows the names and products of the hydrolysis of some of the principal saponins.

Name.	Source.	Products of Hydrolysis.	
		Sugar.	Non-saccharine Product.
Saponin[1] (page 125).	*Saponaria officinalis.*	Dextro-glucose.	Saponegin.
Struthiin.	*Gypsophila* species.	"	"
Quillain (page 128).	*Quillaia saponaria.*	"	"
Senegin.	*Polygala senega.*	"	"
Cyclamin[2] (primulin).	*Cyclamen Europæum.* *Primula officinalis.*	A dextro-glucose.	Cyclamiretin.
Parillin (page 129).	*Smilax* species.	A crystd. glucose.	Parigenin.
Digitonin (page 133).	*Digitalis purpurea.*	A glucose.	Digitoresin; Digitonein.

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[1] According to Dragendorff, the residue obtained by evaporating [illegible] chloroformic or amylic alcohol solution of *Saponaria*-saponin, if moistened with a few drops of concentrated sulphuric acid and exposed to the air, gradually assumes a reddish or reddish-violet coloration, which persists after the addition of two measures of water. *Senegin* is said to act similarly, but to differ in the rapidity with which the colour developes, and to be left as a yellow residue on evaporation of its solution in chloroform. *Digitonin* is said to be distinguished by yielding a fine red colour when heated with dilute sulphuric or hydrochloric acid.

[2] *Cyclamin* or *primulin* is described as crystalline and soluble in water, the solution frothing when agitated. It dissolves readily in dilute spirit, but only sparingly in absolute alcohol, and is insoluble in ether.

The saponins and their congeners may be extracted from the various plants containing them by hot rectified spirit, and are deposited from this solution on cooling.

The saponins are characterised by the strong frothing of their solutions on agitation, and by their power of preventing the deposition of finely divided precipitates. Their aqueous solutions are precipitated by baryta-water and by basic lead acetate. The saponins all undergo hydrolysis by boiling with dilute acids, or by fermentation with emulsin, and some of them are hydrolysed by continued heating with water alone.

Saponin I., $C_{17}H_{26}O_{10}$, from soap-wort, *Saponaria officinalis*, was prepared by Schiaparelli, in 1884 (*Gazetta*, xiii. 422; abst. *Jour. Chem. Soc.*, lxvi. 332), by boiling the dried and coarsely powdered root for three days with strong alcohol. On standing for some days, impure saponin was deposited on the vessel as a copious yellow substance, which was freed from colouring matter by treatment with warm ether-alcohol, followed by solution in alcohol and treatment with animal charcoal. The product, which was still contaminated with about 3 per cent. of mineral matter (chiefly lime), was dissolved in the smallest possible quantity of water; the cold solution precipitated with saturated baryta-water; the resulting barium saponate washed with baryta-water, suspended in water, decomposed by a current of carbon dioxide, the liquid heated to the boiling-point, and filtered; the filtrate concentrated at a gentle heat, and precipitated with excess of alcohol. To remove the last of the barium, the saponin was dissolved in water and dilute sulphuric acid added drop by drop. The filtrate was carefully concentrated and precipitated by ether-alcohol. The product was redissolved and reprecipitated several times, and finally purified by treatment with a quantity of boiling alcohol insufficient for its complete solution. The filtrate evaporated *in vacuo* left flocks of pure saponin, which were washed with ether and dried over sulphuric acid.[1]

The saponin thus prepared is a snow-white, amorphous powder, which has no odour, but excites sneezing when inhaled. It has a disagreeable taste, at first sweet but afterwards sharp and acid, and possesses marked poisonous properties. When heated to 195°, saponin turns brown, and at a somewhat higher temperature evolves an odour of burnt sugar, and leaves a difficultly combustible charcoal.

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[1] Schiaparelli found the saponin thus prepared to contain 52·65 per cent. of carbon and 7·36 per cent. of hydrogen. The formula $C_{32}H_{54}O_{18}$ requires C=52·86 and H=7·44 per cent.; while $C_{17}H_{26}O_{10}$ requires C=52·31 and H=6·67 per cent.

Saponin is very soluble in water, and dissolves in hot rectified spirit, separates again almost entirely on cooling, and is insoluble in absolute alcohol, ether, or benzene. According to Schiaparelli, saponin is insoluble in chloroform, but Dragendorff removes it from its aqueous solution by that solvent. This behaviour would afford a means of purifying saponin far simpler than the tedious method employed by Schiaparelli.

Saponin is precipitated from concentrated solutions by baryta-water and basic acetate of lead. It is not precipitated by picric acid, mercuric chloride, or the general reagents for alkaloids.

Saponin has the lowest optical activity of all known glucosides, the value of $[\alpha]_D$ in a 4 per cent. aqueous solution being $-7{\cdot}30°$. The aqueous solution of saponin, if not more dilute than 1 : 1000, froths very strongly and persistently on shaking, and has a remarkable power of dissolving salts (*e.g.*, barium sulphate) insoluble in water. A solution of saponin, mixed with lead acetate and treated with sulphuretted hydrogen, yields on filtration a clear black liquid, from which lead sulphide is precipitated on adding alcohol. A boiling aqueous solution of saponin dissolves 10 per cent. of barium carbonate, and the dissolved barium is not perfectly precipitated by sulphuric acid.

Saponin acts as a powerful poison on the lower animals. Its effects on a kitten, when injected hypodermically, were observed by A. Wynter Blyth to be more rapid respiration, lethargy, and signs of general muscular weakness, with ultimate asphyxia. A *post-mortem* examination showed fulness on the right side of the heart and intense congestion of the intestinal canal, the appearance of the stomach and other organs being normal.

When saponin is boiled with a dilute mineral acid it suffers hydrolysis, being converted into a glucose and an insoluble substance. The glucose is dextrogyrate ($\alpha_D = +52{\cdot}48°$) and fermentable; but, as it has not hitherto been obtained crystallised, its identity with ordinary dextrose is not fully established. The insoluble body simultaneously formed has been named and formulated differently by various observers; but it is preferably called sapogenin, and if Kobert's formula for saponin be accepted, its formation is expressed by the following equation[1] :—

$$2C_{17}H_{26}O_{10} + 2H_2O = 3C_6H_{12}O_6 + 2C_8H_{10}O_2.$$

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[1] Rochleder ascribes to saponegin the formula $C_{14}H_{22}O_2$, and represents its formation from saponin by the formula :—

$$C_{32}H_{54}O_{18} + 2H_2O = 3C_6H_{12}O_6 + C_{14}H_{22}O_2.$$

Schiaparelli expresses the hydrolysis of saponin as follows :—

$$2C_{32}H_{54}O_{18} + 3H_2O = 4C_6H_{12}O_6 + C_{40}H_{66}O_{15}.$$

Sapogenin is described as crystallising from alcohol in concentric groups of needles, insoluble in water, very difficultly soluble in cold alcohol, more readily in hot, and soluble in ether. It may be obtained crystallised by spontaneous evaporation of its alcoholic solution. Saponegin dissolves in dilute caustic potash, and with stronger alkali deposits flocks of a potash compound. Fused with caustic potash, it yields much acetic acid, some butyric acid, and a crystalline isomer of sapogenin melting at 128° C.

Saponin is precipitated from fairly concentrated solutions by basic lead acetate, and with solutions of caustic potash, strontia, and baryta yields sparingly soluble precipitates of definite composition. The baryta-compound, said by Stütz to contain $2C_{19}H_{30}O_{10},Ba(OH)_2$, has been employed by Christophsohn and Otten for the determination of saponin, as follows:—10 grammes weight of the substance is boiled three times in succession with water, the decoctions strained, evaporated to a small bulk, precipitated with alcohol, and filtered. The precipitate is exhausted with boiling alcohol, which is added to the filtrate, and the alcohol distilled off. The residue is dissolved in water and the concentrated solution treated with saturated baryta-water. The precipitate is collected on a weighed filter, washed with saturated baryta-water till the washings are colourless, and dried first at 100° and then at 110° C. After weighing, the compound is converted into barium carbonate by simple ignition, or into the sulphate by moistening with sulphuric acid and again igniting. The weight obtained is calculated to BaO, and deducted from the weight of the dried precipitate, the difference being the saponin in the substance operated on. When the substance to be examined contains much starch (*e.g.*, the seeds of *Agrostemma githago*), it should be exhausted with boiling alcohol, the liquid filtered hot, and the alcohol distilled off. The residue is treated with ether to remove fatty oil, dissolved in water, and the solution precipitated with baryta as before.

Where there is any doubt as to the purity of the precipitate of saponin-baryta obtained, it may be dissolved in water acidulated with hydrochloric acid, and the baryta removed by the cautious addition of dilute sulphuric acid. The filtrate is boiled for an

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He calls the insoluble product "saponetin," and describes it as micro-crystalline and soluble in alcohol, but insoluble in water or ether.

Hesse substantially adopts Rochleder's view, and considers that the hydrolysis of saponin, with formation of saponegin, saponetin and saporetin (senegenin), can be very readily expressed on the assumption that saponin has the formula $C_{32}H_{52}O_{17}$, thus differing only by the elements of water from Rochleder's formula.

hour, and the saponegin which separates filtered off, washed, and, together with the filter, boiled with alcohol of ·855 specific gravity. The filtered spirituous solution is evaporated, and the residue dried at 110° C. 35·8 parts of saponegin thus obtained represent 100 parts of saponin. Christophsohn found this modified process to give results which agreed well with those deduced from the weight of the baryta precipitate, and has published the following figures:—

	Saponin; per cent.	
	Baryta method.	Saponegin method.
Quillaia saponaria (bark), . . .	8·67	8·82
Gypsophila struthium (root), . .	14·59	15·00
Do. do., . . .	13·31	13·20
Saponaria officinalis (root), . . .	4·78	5·09
Agrostemma githago (ripe seeds), .	6·67	6·51

Otten found, by weighing the baryta precipitate, from 1·21 to 3·47 per cent. of saponin in the various species of sarsaparilla.

QUILLAJIC ACID, $C_{19}H_{30}O_{10}$, was prepared by R. Kobert (*Chem. Centralb.*, 1888, 927; *Journ. Chem. Soc.*, lvi. 55) from the bark of *Quillaja saponaria*, by precipitating the aqueous extract with neutral lead acetate. The solution was freed from lead, the filtrate evaporated almost to dryness, and the residue taken up with hot absolute alcohol. From this solution the colouring matter was precipitated by chloroform. The quillajic acid subsequently separated in white flakes, soluble in water and alcohol, but insoluble in ether. On treatment with strong sulphuric acid it becomes dark red. The sodium salt of quillajic acid acts as a severe caustic on the tongue and throat, and the smallest particles coming in contact with the nose and throat cause violent sneezing and coughing. Brought in contact with the eyes, it causes severe pain, flow of tears, and swelling of the lids. Injected into the blood, the sodium salt proves fatal, causing cramp and paralysis of the respiratory organs and brain; while, on the other hand, it may be taken by the stomach without injury to the extent of 500 times the quantity which proves fatal when injected into the blood.

Kobert states that the saponin of commerce, as also other specimens of saponin, consists of an almost inactive, non-poisonous modification of quillajic acid.

Stütz (*Annalen*, ccxviii. 250) has described tetra- and pent-cetyl-derivatives of saponin from quillaia bark. The former body, btained by heating saponin with acetic anhydride for half an hour, was a white powder, having the composition $C_{19}H_{26}O_{10}(C_2H_3O)_4$. t melted at 159° to 162°; was insoluble in water, but readily oluble in alcohol, ether, and glacial acetic acid; and on heating with baryta-water was decomposed with regeneration of saponin.

THE GLUCOSIDES OF SARSAPARILLA have been recently investigated by W. von Schulz, who concludes that Dragendorff's smilacin or sarsaparilsaponin, $C_{20}H_{32}O_{10}$, the sarsasaponin, $C_{22}H_{36}O_{10}$, discovered by himself, and Flückiger's parillin, $C_{26}H_{44}O_{10}$, are three homologous compounds all belonging to the series having the general formula $C_nH_{2n-8}O_{10}$. These three substances all split up into sarsasapogenin and one or more molecules of glucose on boiling with dilute acids.

A reaction of smilacin has been described by Serena, who states that when it is treated with a few drops of strong sulphuric acid and a very small quantity of a dilute solution of ferric chloride added, with the aid of a gentle heat, a deep orange-yellow coloration is produced, changing to reddish-brown with a violet eflection, then violet, bluish-green, and finally light violet. Digitalin, when similarly treated, is said to give a brownish-red coloration, gradually acquiring a violet tinge and on addition of water changing to greenish-yellow.

Physiological experiments with the glucosides of sarsaparilla show that in pharmacological value they belong to the same group as sapotoxin. Sarsasaponin appears to be most active in subcutaneous injections, 50 milligrammes per kilogramme of body-weight proving fatal to dogs and cats, while the respective lethal doses of parillin and sarsaparilsaponin are three or four times as large (*Pharm. Jour.*, [3], xxiii. 6).

Parillin, $C_{26}H_{44}O_{10}$, is described by Flückiger as not appreciably soluble in cold water, but dissolved by about twenty parts at the boiling-point, and as being taken up more readily by alcohol of about 0·83 specific gravity than by either weaker or stronger spirit. With strong sulphuric acid it behaves like saponin, and when boiled with 10 per cent. sulphuric acid is hydrolysed, with production of a green fluorescence and formation of glucose and arigenin. A similar fluorescence is developed when hydrochloric cid gas is passed into a solution of parillin in a mixture of alcohol nd chloroform.

SAPOTIN is the name given to a glucoside extracted by G. Michaud (*Amer. Chem. Journ.*, xiii. 572) from the seed contained n the Sapodilla plum, the fruit of *Achras Sapota*. It was obtained in minute crystals, melting at 240°. In alcoholic solution they are lævo-rotatory, $[\alpha]_D = -32{\cdot}11°$. Sapotin is very soluble in water, less so in alcohol, and insoluble in ether, benzene, or chloroform. Lead acetate forms a gelatinous precipitate in an aqueous solution of the substance, the precipitate being soluble in excess of the reagent. The formula of sapotin is stated to be $C_{29}H_{52}O_{20}$, and when boiled with dilute sulphuric acid it yields glucose and sapotiretin, $C_{17}H_{32}O_{10}$, a body insoluble in water, but very soluble in alcohol.

Glucosides of Digitalis.

The leaves and seeds of the purple foxglove (*Digitalis purpurea*) contain several closely-allied active principles, some at least of which are of a glucosidal nature.[1]

The chemistry of *Digitalis* has formed the subject of numerous researches, but the composition and properties of the bodies isolated are still far from being accurately known; and, to increase the confusion, French and German investigators apply the same name to compounds of a different nature.

According to the researches of Schmiedeberg (*Pharm. Jour.*, [3], v. 741), the seeds and leaves of *Digitalis purpurea* contain a preponderating amount of digitonin, a glucoside of the saponin class (page 123). They contain, in addition, three other substances which possess the character of acting on the heart; namely, crystallisable digitoxin, which is not glucosidal, and the two amorphous glucosides digitalin and digitalein. Besides these, there has been isolated from digitalis an inert, crystalline body called digitin, $C_4H_9O_2$.

H. Kiliani (*Archiv. der Pharm.*, ccxxx. 250; *Pharm. Jour.*, [3], xxii. 1061; [4], i. 29) substantially endorses Schmiedeberg's conclusions, though he doubts the homogeneous nature of Schmiedeberg's digitalein, and believes digitin to be simply digitonin.

The other constituents of digitalis are not characteristic. They include chlorophyll, mucilage, albuminous matters, various salts, and inosite. The occurrence of the last substance was observed by Marmé.

For an improved method of preparing pure digitalin from the commercial article called by that name, see Schmiedeberg, *Pharm. Jour.*, [3], v. 744; [4], i. 29.

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[1] A preparation intended to represent the active principles of *digitalis* was described in the British Pharmacopœia of 1867, but was of very indefinite composition and consequent uncertain activity. Hence it has been omitted from more recent editions.

Digitalin. According to H. Kiliani, pure digitalin forms a white amorphous powder, or soft white grains, which remain unchanged when heated to 200°, at 210° begin to aggregate, and towards 217° melt, becoming yellow. When treated with water, the particles of digitalin swell up and dissolve slightly (1 : 1000), giving a solution which foams strongly on agitation, and is remarkably prone to become mouldy. Digitalin is dissolved sparingly by cold proof-spirit (1 : 100), but in considerable amount by hot rectified spirit or absolute alcohol. When a minimum of alcohol is used, the solution on cooling becomes almost solid from the separation of a thick magma of granules, which under the microscope appear of very uniform size, but entirely destitute of crystalline structure. This behaviour is stated by Kiliani to be a characteristic peculiarity of digitalin, and a valuable criterion of its purity. In presence of only a small percentage amount of the glucosides associated with it in digitalis it is impossible to effect the separation of digitalin from solution in the form of granules. When it also contains some digitonin, and 85 per cent. spirit is used for its solution, crystals will be found among the granules deposited from the solution after cooling. Kiliani states that these impurities of digitalin may be detected with greater accuracy by the following tests :—

A few granules, when mixed with about 2 c.c. of a 10 per cent. solution of caustic potash, should retain their whiteness for at least one minute. The minutest admixture of amorphous glucosides rapidly causes an intense yellow coloration.

When digitalin is made into a thin paste with water, and for every 100 parts of water 22 parts of amylic alcohol are added, distinct crystalline warts will become apparent on leaving the mixture for twenty-four hours in a closed flask, if digitonin be present even in very minute proportion.

Digitalin is very sparingly soluble in ether or chloroform.[1] When ether is gradually added to the moderately dilute solution in absolute alcohol, the liquid suddenly becomes turbid, and a copious deposition of granules of digitalin subsequently occurs. If the liquid be decanted and more ether added, the same effect may be produced a second and third time.

Dragendorff states that "digitalin" is extracted from acidulated aqueous liquids by agitation with warm benzene.

Contrary to previous observers, Kiliani finds the taste of digitalin but slightly bitter, and believes the intense bitterness and very disagreeable taste previously ascribed to digitalin to be due to admixture with the amorphous glucosides associated with it in digitalis.

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[1] The very sparing solubility of digitalin in chloroform prohibits the presence of any considerable proportion in the "digitalin" of the French Codex, which is required to be completely soluble in chloroform.

Digitalin dissolves in strong hydrochloric or sulphuric acid with golden-yellow colour, and in the latter case the colour rapidly changes to a blood-red. On adding to the solution, while still yellow, a drop of nitric acid, ferric chloride or bromine-water, a brilliant purple coloration is produced. The colour is very fugitive, and excess of the oxidising reagent should be carefully avoided; and Kiliani states that the reaction is most permanent and delicate when the minute trace of nitric acid apt to be present in the sulphuric acid used is relied on to effect the oxidation. A preferable plan is to expose the solution of digitalin in strong sulphuric acid to the vapours of bromine.

Schmiedeberg obtained by the analysis of digitalin numbers corresponding to the empirical formula $C_5H_8O_2$. This analysis is confirmed by Kiliani, who, however, from a study of the products of its hydrolysis, prefers for digitalin the formula $C_{29}H_{46}O_{12}$, which only differs from $6C_5H_8O_2$ by CH_2.

When digitalin is heated with dilute hydrochloric acid, an insoluble resinous substance almost immediately separates. This is called by Schmiedeberg digitaliresin, but Kiliani regards the product thus obtained as an indefinite substance containing unaltered granules of digitalin. By operating in alcoholic solution Kiliani avoids the separation of resin, and finds that digitalin is, under these conditions, split up very definitely into digitaligenin, glucose, and digitalose, according to the following equation:—

$$\underset{\text{Digitalin.}}{C_{29}H_{46}O_{12}} + H_2O = \underset{\text{Digitaligenin.}}{C_{16}H_{22}O_2} + \underset{\text{Glucose.}}{C_6H_{12}O_6} + \underset{\text{Digitalose.}}{C_7H_{14}O_5}.$$

To effect this reaction, Kiliani treats digitalin with 8 parts of 50 per cent. alcohol, adds 2 parts of fuming hydrochloric acid (sp. gr. 1·19), and heats the mixture for half an hour at 100° under a reflux condenser. The liquid rapidly darkens, but does not deposit more than traces of resinous matter. On cooling, abundance of digitaligenin separates in warty masses composed of brilliant acicular crystals. The crystallisation is facilitated by rubbing the sides of the containing vessel. When pure digitalin has been used, the digitaligenin separates readily in fine crystals; but in presence of some of the other glucosides of digitalis it cannot be made to crystallise at all, or only by very tedious operations. When washed with 50 per cent. alcohol and dried, the yield of digitaligenin from pure digitalin is constantly 30 per cent. If the filtrate be diluted with an equal measure of water, repeatedly shaken with ether, and the separated ether shaken with very dilute soda to remove hydrochloric acid, a further quantity of digitaligenin is obtainable. When recrystallised from the least possible quantity of 93 per cent. alcohol, digitaligenin forms colourless brilliant needles, melting at 210°–212°. It is insoluble in water, sparingly soluble in ether free from alcohol, and readily soluble in hot alcohol. Digitaligenin appears to be physiologically inactive. With strong sulphuric acid it gives the same reaction as digitalin.

In the filtrate from the digitaligenin, Kiliani obtained evidence of the presence of ordinary glucose and of digitalose, a sugar-like body allied to rhamnose, but uncrystallisable. He attributes to digitalose the formula $C_7H_{12}O_5$.

Digitalin is a powerful heart-poison. The maximum medicinal dose for human beings is 0·00025 gramme. Administered to frogs in doses of 0·0005 gramme it produces systolic stoppage of the heart in fifteen to twenty minutes. The effect of digitalis is similar, but hurtful and so-called cumulative action is apt to occur, manifesting itself especially in derangement of the stomach. This secondary action is probably due to digitonin, which is the active principle occurring most abundantly in digitalis, and has been found to produce very severe local inflammatory symptoms.

DIGITONIN forms fully one-half of the mixed glucosides from digitalis seed, and the principal portion of German "digitalin," from which it is prepared by extraction with and crystallisation from 85 per cent. alcohol. The solution should be kept at 45° for six hours and then allowed to cool slowly, when the digitonin is deposited in a form much more readily drained by the pump (Kiliani, *Arch. Pharm.*, ccxxxi. 460. See also Schmiedeberg, *Pharm. Jour.*, [3], v. 742; and Kiliani, *Pharm. Jour.*, [4], i. 29). Digitonin softens at 225° (about 250°, according to Houdas) and melts completely at 235°. The crystals dissolve sparingly in cold water, more readily in hot, to an opalescent solution, which froths on agitation. On evaporation, the solution yields only a gummy mass, all attempts to crystallise digitonin from water being hitherto unsuccessful. The aqueous solution is lævo-rotatory ($[a]_D = -49·25°$), and is precipitated by tannin, ammoniacal lead acetate, and baryta-water. Digitonin is only slightly soluble in absolute alcohol, and still less so in ether, chloroform, or petroleum-spirit. It is almost completely precipitated by adding ether to its alcoholic solution.

When amylic alcohol is added to an aqueous solution of digitonin, the glucoside is rapidly separated in a crystalline form. If a hot mixture of amyl and ethyl alcohols be used, the solution deposits, on cooling, long nacreous laminæ, which contain amyl alcohol and water of crystallisation. Similar crystalline compounds of digitonin with other alcohols and with phenol have been described by J. Houdas. (See further, Kiliani, *Pharm. Jour.*, [3], xxiv. 45.)

Digitonin dissolves in strong sulphuric acid with red colour, an addition of a drop of bromine-water intensifying the reaction but not changing it to violet. Sulphuric acid diluted with its own volume of water produces a yellowish coloration in the cold, changing to red and finally to black on heating. Concentrated hydrochloric acid gives a colourless solution, which after a time, or on heating, turns yellow and then violet-red, with a slight greenish fluorescence.

According to Kiliani (*Ber.*, xxiv. 339), digitonin has the formula $C_{27}H_{46}O_{14}+5H_2O$, and when boiled for some time with strong alcohol and hydrochloric acid splits up into equal molecules of dextrose, galactose, and digitogenin, the last body separating in warty masses on cooling:—

$$\underset{\text{Digitonin.}}{C_{27}H_{46}O_{14}}+H_2O=\underset{\text{Dextrose.}}{C_6H_{12}O_6}+\underset{\text{Galactose.}}{C_6H_{12}O_6}+\underset{\text{Digitogenin.}}{C_{15}H_{24}O_3}.$$

J. Houdas (*Compt. rend.*, 1891, page 648) attributes to digitonin (which he prefers, with Nativelle, to call digitaleïn)[1] the tentative formula, $C_{31}H_{52}O_{17}$, which agrees with that of Schmiedeberg, and states that the substance he examined was converted on treatment with very dilute sulphuric acid into two new crystalline glucosides, without the formation of any glucose; though he does not deny that the bodies obtained by Kiliani may not have been the final products.

DIGITOXIN, $C_{21}H_{32}O_7$ (?), is obtained by exhausting digitalis leaves with water and then with dilute alcohol, precipitating the tincture with lead acetate, concentrating, crystallising, &c. The product is purified by washing with a dilute solution of sodium carbonate, followed by petroleum-spirit, and then recrystallised from strong alcohol. Digitoxin crystallises in pearly plates or needles, which melt at 240°. It is quite insoluble in water, to which it does not even impart a bitter taste. It is also insoluble in benzene and petroleum-spirit, and only very sparingly soluble in ether; but dissolves in chloroform and in alcohol, the solution in the latter menstruum being intensely bitter.

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[1] According to Schmiedeberg, digitonin and digitaleïn are distinct compounds, the latter only being soluble in chloroform, while the solutions of both substances froth on agitation. Digitaleïn is stated to react with sulphuric acid in the same manner as digitalin, which it also resembles in its physiological action. Kiliani regards the existence of digitaleïn as highly questionable.

Digitoxin does not give the colour-reaction of digitalin with strong sulphuric acid. Warmed with strong hydrochloric acid, it gives a yellow or greenish coloration, which is one of the reactions ascribed to the "digitaline crystallisé" of the French Codex.

Digitoxin is not a glucoside. When warmed in alcoholic solution with dilute acids, it is decomposed with great facility, forming toxiresin, without production of glucose. Toxiresin is an uncrystallisable, yellowish substance, readily soluble in ether. It is a very powerful muscle-poison.

Digitoxin is the most poisonous of the constituents of digitalis, its toxicity being so great as to render it unsuitable for medical use.

DIGITIN, $nC_4H_9O_2$, is described as crystallising in stellate groups of needles or in small plates. It is not dissolved by chloroform or benzene, but is extremely soluble in ether. It is insoluble in water, but is dissolved by alkalies, acids precipitating it from the alkaline solution.

Strong sulphuric acid dissolves digitin with brownish-yellow colour, which becomes purple-red on exposure to air; addition of water turning the colour to green. In hydrochloric acid, digitin is insoluble, but dissolves in nitric acid without coloration.

Digitin does not reduce Fehling's solution till after boiling with dilute acid, when it gives a strong glucose reaction.

Kiliani regards Schmiedeberg's digitin as simply digitonin.

COMMERCIAL DIGITALIN.—E. Merck (1886) has given the following description of the principal brands of commercial digitalin:—

1. "*Digitalin. pur. pulv.*," or "German digitalin," consists principally of digitaleïn, with some digitonin and digitalin. Digitaleïn, in consequence of its ready solubility in water, is not cumulative in its action, and causes no irritation when subcutaneously injected (compare page 134). It is freely soluble in alcohol, but insoluble in ether and in chloroform.

2. "*Nativelle's crystallised digitalin.*" Physiologically extremely active. In fine white needles, bitter in taste, insoluble in water, ether, or benzene; freely soluble in chloroform. Consists almost entirely of digitoxin, and is cumulative in its action. Typical of "French digitalin." Corresponds with the "digitaline crystallisé" of the French Codex.

3. "*Homolle's amorphous digitalin.*" A white or yellowish-white amorphous bitter powder, slightly soluble in water and ether, freely soluble in 90 per cent. alcohol and in chloroform. It consists principally of digitalin, with some digitoxin. Corresponds

with the "Digitaline amorph." of the French Codex and Belgian Pharmacopœia.

4. "*Digitalin. pur. pulv.* (*Merck*)." A yellowish-white powder, corresponding in its properties with No. 1. Merck also prepares (5) a "crystallised digitalin," identical with digitin, difficultly soluble in water, more readily in alcohol, and insoluble in ether or chloroform; as well as (6) "digitoxin," the most poisonous of all the digitalis bodies, cumulative in action, crystallising from alcohol in concentrically grouped needles, freely soluble in chloroform and alcohol, but sparingly soluble in ether. The amorphous "digitalins" of the Codex and the Belgian Pharmacopœia are said by Merck to consist essentially of digitalin,[1] with some digitoxin, and to correspond to Homolle's amorphous preparation (No. 3).

Preparations 2, 3, and 6 of Merck's list are insoluble in water but soluble in chloroform, whilst Nos. 1 and 4 are soluble in water but insoluble in chloroform. Fouquet considers the former class should exclusively be used in medicine.

H. Kiliani (*Archiv. der Pharm.*, ccxxx. 250; and *Pharm. Jour.*, [3], xxii. 1061) considers that digitoxin is not well suited for therapeutic use, owing to its complete insolubility in water, while digitaleïn is of an indefinite nature; but that true digitalin is, when pure, very suitable for therapeutic use, the only drawback being its amorphous character.

"Commercial digitalin" is stated to give an absorption-spectrum showing a dark line in the blue, coincident with the Fraunhöfer line F.

Toxicology of Digitalis.—Plants watered with a solution of digitalis wither and die. Fish placed in a weak infusion of the plant die in spasm, with the ventricle of the heart contracted. Poisonous doses of digitalis given to birds, dogs, and other animals occasion active intestinal irritation, with watery or bloody dejections.

When animal or vegetable matters are submitted to the usual process for the isolation of glucosides, &c., the digitalis principles mostly pass into the acid benzene, ether, or chloroform extract. The most toxic principles, digitalin and digitoxin, are very readily altered by acids, but as the products of their change are more soluble in ether than the original substances, and give much the same colour-reactions, it may sometimes be an advantage to allow the change by acids to occur. The decomposition-product of digitoxin is equally poisonous with the parent substance. The most serviceable tests for the digitalis principles, when isolated, are the colour-reactions already described (pages 132, 134).

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[1] Kiliani states that the principles extracted from the leaves of digitalis are quite different from those present in the seeds, and that digitalin does not occur in the leaves. If this be true, the so-called "digitalin" of the Belgian Pharmacopœia, which is prepared from the leaves, can contain no true digitalin.

H. Brunner (*Berichte*, 1873, page 96) states that the acid ethereal extract from digitalis gives a red colour when its solution is mixed with a dilute aqueous solution of dried ox-bile, and then sufficient sulphuric acid added to raise the temperature to 70°. By this reaction, Brunner detected the presence of 0·3 gramme of foxglove leaves in 180 c.c. of water, and he states that it serves to distinguish digitalin from all substances except those which give a red colour with sulphuric acid.

Glucosides having physiological effects closely analogous to those exhibited by *digitalis* exist in several other plants. Among them may be mentioned the following:—

Helleborin, $C_{36}H_{42}O_6$, and *Helleboreïn*, $C_{26}H_{44}O_{15}$, in the root of the Christmas rose and other species of hellebore (see page 68).

Adoninin, contained in the root of *Adonis vernalis*.

Oleandrin and *Neriin*, in the leaves of the oleander. The former substance is alleged to have basic properties, though free from nitrogen, while neriin is not improbably identical with digitaleïn.

Thevetin, $C_{54}H_{48}O_2$ (?), has been isolated from *Thevetia nereifolia*, and is possibly identical with the glucoside of *Cerbera odallam*.

Euonymin has been isolated from the resin of *Euonymus atropurpureus*.

Scillitoxin is a powerful heart-poison, apparently of glucosidal character, contained in the bulbs of the common squill, together with *scillipicrin* and *scillin*, of inferior physiological action (see E. Merck, *Pharm. Jour.*, [3], ix. 1038). A remarkable case of poisoning by squills has been investigated by E. B. Truman (*Pharm. Jour.*, [3], xvi. 828, 832; xvii. 227).

Strophanthin is described at length on page 139, *ouabaïn* on page 140, and *antiarin* on page 141.

Glucosides of Strophanthus.

A poisonous, readily-decomposable glucoside called strophanthin is contained in various parts of *Strophanthus Kombé*, a climbing plant belonging to the order *Apocynaceæ*.[1] The seeds of *Strophanthus*, which are the part of the plant richest i glucoside, are employed by the natives of Central and Wes Africa for the preparation of the arrow-poison called *komb ineé*, or *onaje*.

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[1] Numerous papers on the botany and physiology of *Strophanthus* will be found in the *Year-Book of Pharmacy* during the past decade.

A valuable paper on "The Strophanthus Seeds of Commerce," by E. M. Holmes, will be found in the *Pharm. Jour.*, [3], xxiii. 867, 927.

A specimen of *Strophanthus* seed, analysed by T. R. Fraser (*Year-Book Pharm.*, 1889, p. 450), gave the following results:—

Water (loss at 100° C.),	6·70	per cent.
Petroleum-ether extract (chiefly fat),	31·81	"
Ether extract (resin, chlorophyll, &c.),	0·85	"
Rectified spirit extract (strophanthin, mucilage, resin),	8·94	"
Water extract, { Mucilage,	7·35	"
Albumin,	1·95	"
Insoluble and undetermined constituents,	38·89	"
Mineral matters,	3·51	"
	100·00	

Other analyses by Fraser showed that the ether-extra consisting mainly of fat with a small quantity of chloroph and resin, amounted to about 34 per cent.; while the alcoho extract, containing the active principle, was about 9·5 per ce It has been shown by Elborne, Merck, and others, that presence of the fat ether dissolves some of the strophanthin, fact which should not be lost sight of in judging the strength an alcoholic extract of strophanthus.

A specimen of strophanthus seeds examined by W. Elbor (*Pharm. Jour.*, [3], xvii. 746), gave, after drying to remove wat —Fixed oil extracted by petroleum ether, 20·8 per cent.; chlor phyll and fat extracted by ether, 0·9; bitter glucoside extract by absolute alcohol, 1·5; further bitter glucoside extracted water, 2·9; albuminous matters soluble in water, 19·6; and i soluble residue, 54·3 per cent.

To isolate the glucoside of strophanthus, the alcoholic extr of the seeds should be dissolved in a minimum quantity of wat and precipitated by a solution of tannin. The washed precipit is mixed with sufficient moist hydroxide of lead to combine wi the tannin employed, the mixture allowed to stand for so days, and, when dry, extracted in succession with rectified a with proof spirit. The excess of lead is removed by passing stream of carbon dioxide[1] through the alcoholic liquid for so days, when the filtered liquid is evaporated to dryness, the residue exhausted with rectified spirit, and the strophanthin precipitated from the solution by the addition of ether. The product is purified by re-solution in alcohol and evaporation *in vacuo*, supplemented by drying over sulphuric acid.[1]

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[1] Strophanthin is extremely susceptible to the action of acids, and e sulphuretted hydrogen is liable to cause some decomposition.

STROPHANTHIN forms a pale yellowish amorphous powder, or a mass of microscopic crystalline plates or nucleated spangles, which are transparent while wet but become opaque on drying. According to Arnaud (*Compt. rend.*, cvii. 179), the crystals of strophanthin soak up and retain water with great facility, forming a hydrate which parts with its water in a dry vacuum, or when dried in the air; or if heated in a drying chamber, melts below 100°, and leaves anhydrous strophanthin in an uncrystallisable condition; but if the strophanthin be previously rendered anhydrous in a vacuum, it can be heated to 110° without alteration. Strophanthin darkens at about 146°, becomes pasty at 165°, and melts at 172·5° (185°, according to E. Merck).

Strophanthin has an intensely bitter taste, and is extremely poisonous.

Strophanthin dissolves very readily in water and aqueous alcohol; less readily (1:55) in absolute alcohol, amylic alcohol,[2] or acetone (1:300); and is practically insoluble in chloroform, ether, benzene, petroleum-ether, or carbon disulphide. Ether or chloroform containing alcohol or water dissolves a sensible quantity of strophanthin, as does ether containing the fixed oil of strophanthus in solution. From alkaline solutions saturated with common salt, strophanthin is said to be extracted by ether-chloroform. Solutions of strophanthin in rectified spirit and in amylic alcohol are precipitated by the addition of chloroform, ether, petroleum-ether, or carbon disulphide. When ether is added to a very dilute alcoholic solution, and the faintly turbid liquid allowed to stand for a few days, stellar groups of crystals of strophanthin are deposited.

Strophanthin differs from the great majority of glucosides in being *dextro*-rotatory, the value of $[\alpha]_D$ for a 2·3 per cent. solution in water being +30°.

The formula of strophanthin cannot be regarded as definitely established. The percentage composition first published by T. R. Fraser (*Pharm. Jour.*, [3], xviii. 69), to whom the most accurate knowledge of strophanthin is due, was C, 55·97; H, 7·75; and O, 36·28; from which he deduced the formula $C_{20}H_{34}O_{10}$, suggesting a close connection between strophanthin and the saponins (page 123). At a later date (*Year-Book Pharm.*, 1889, page 463), as the average of several concordant analyses, Fraser found C, 55·43; H, 7·56; and O, 37·01; corresponding to the formula $C_{16}H_{26}O_8$. Arnaud (*Compt. rend.*, cvii. 1162) attributes to strophanthin the formula $C_{31}H_{48}O_{12}$, and regards it as the immediate higher homologue of ouabain.[1]

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[1] The yield from the seeds is only 4 to 6 parts per cent., but the alcoholic extract gives 65 per cent. of its weight.

[2] Amylic alcohol extracts strophanthin from its aqueous solution, but the fact is of little value for isolating it from strophanthus, owing to the formation of an obstinate emulsion.

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[1] OUABAIN, $C_{30}H_{46}O_{12}$, is the poisonous glucoside contained in the root and wood of the *Acokanthera Ouabaio*, from which the Comalis on the east coast of Africa prepare their arrow-poison. Ouabain forms slender transparent needles, having a characteristic rectangular form, or white nacreous laminæ, which are tasteless and odourless. At 180° it softens, and melts completely at 200°. Ouabain is almost insoluble in cold water, but dissolves readily on boiling, and has a great tendency to form supersaturated solutions. It crystallises from water with $7H_2O$, of which $6H_2O$ are lost at 100, but the remaining molecule is not completely expelled below 130°. Ouabain dissolves readily in aqueous alcohol, but is almost insoluble in absolute alcohol, and is quite insoluble in ether or chloroform. The concentrated aqueous solution is neutral to litmus, gives a precipitate with tannin, and has a specific rotation of −34° for the D ray. When boiled with dilute acids, ouabain yields a reducing sugar. Boiled with baryta-water, ouabain yields a barium-derivative, which, after drying at 100°, has the composition $Ba(C_{30}H_{47}O_{12})_2$. Arnaud, to whom the above description of ouabain is due (*Compt. rend.*, cxx. 1011), states that it has no poisonous effect when introduced into the stomach, but that when administered by subcutaneous or intervenous injection, it acts on the heart and causes death. 0·002 gramme will kill a dog of 12 kilogrammes weight in a few minutes. E. Gley (*Compt. rend.*, cvii. 348) states that the characteristic effect both of ouabain and strophanthin is the rapid arrest of the heart in systole. 0·025 milligrm. of ouabain produces this effect on a frog in six minutes, while the same quantity of strophanthin requires twelve minutes. To the rabbit, ouabain is twice as poisonous as strophanthin, to a dog twice, and to a guinea-pig four times as poisonous; besides which strophanthin is always less rapid in its action. Ouabain is recommended medicinally in half the doses of strophanthin, in cases where both strophanthus and digitalis fail.

Arnaud (*Compt. rend.*, cvii. 1162) has isolated from the seeds of *Strophanthus glabre*, a tree growing in the Gaboon, 4·7 per cent. of a glucoside which he regards as identical with ouabain.

TANGHININ, $C_{27}H_{40}O_8$, is the active principle of tanghin, the judicial poison of the Malgaches, which is extracted from the kernel of the fruit of *Tanghinia venenifera*, one of the *Apocynaceæ*. Tanghinin forms colourless rhombic crystals, becoming pasty at 170°, and melting at about 182°. It is almost insoluble in water, but when left in contact with it swells up and forms a thick mucilage containing microscopic crystals in suspension. Tanghinin is readily soluble in strong alcohol, and moderately soluble in ether. A

The aqueous and alcoholic solutions of strophanthin have an acid reaction, but no salt of strophanthin appears to have been prepared.

saturated alcoholic solution at 20° C. has an optical activity of $[\alpha]_D = -67°$. Tanghinin is a cardiac poison resembling ouabain and strophanthin. It gives no colour-reactions, and does not appear to be a glucoside (Arnaud, *Compt. rend.*, cviii. 1255).

The arrow-poison of the Wa Nyika and other tribes of east equatorial Africa has been examined by Fraser and Tillie (*Pharm. Jour.*, [3], xxiii. 937), who attribute to the active principle the formula $C_{30}H_{52}O_{14}$, and regard it as closely analogous to, if not actually identical with, ouabain.

A review of the arrow-poisons of the genus *Acokanthera* has been published by E. M. Holmes (*Pharm. Jour.*, [3], xxiv. 41).

Antiarin is the poisonous glucoside which is the active principle of the arrow-poison prepared from the *Antiaris toxicaria* or Upas Antjar of Java. Antiarin is isolated by treating the inspissated milky juice of the plant with petroleum-ether to remove fatty matters, &c., and then extracting the residue with absolute alcohol. The alcohol is evaporated, the residue taken up with water, the solution precipitated with lead acetate, the filtrate freed from lead and evaporated. According to de Vry and Ludwig the yield is about 4 per cent. of the juice.

Antiarin is represented by the formula $C_{14}H_{20}O_5 + 2$ aqua. If this formula be doubled it becomes $C_{28}H_{40}O_{10}$, and the analogy of antiarin to ouabain is apparent. Antiarin crystallises in shining colourless plates, which melt at 220·6°. It dissolves in about 250 parts of cold or 27 of boiling water, and is soluble in alcohol. It is insoluble in benzene and requires 2800 parts of ether for solution. The aqueous solution of antiarin is neutral to litmus and is not precipitated by metallic salts. On warming with dilute acids it yields glucose and a resinous body. With concentrated sulphuric acid it gives a yellowish-brown coloration.

According to Bettink, if 1 c.c. of a 5 per cent. solution of sodium carbonate be boiled with three drops of a cold saturated solution of picric acid, the yellow colour will not perceptibly change, but if as little as 0·0001 gramme of antiarin be added, the colour will become orange-red, probably owing to the reducing action of the antiarin and consequent formation of picramic acid. Æsculin, amygdalin, digitalin, and picrotoxin are said to give no similar reaction.

Antiarin is a highly toxic substance, acting essentially as a muscle and heart poison, and closely resembles digitalis in its physiological effects. Any attempt at detection should be conducted on the lines of the process used for its extraction.

The intensely active arrow-poison used by the pigmies met with by H. M. Stanley in Central Africa is compounded from five plants. Its toxic action is believed by E. M. Holmes to be due to erythrophlœine and strychnine (*Pharm. Jour.*, [3], xxi. 917).

The Indian arrow-poison known as curare (Part ii. page 387) is an extract prepared from the bark of *Strychnos toxifera*, together with other vegetable extracts (see J. Moss, *Pharm. Jour.*, [3], viii. 121).

The arrow-poison used by the Indians of New Granada has been investi-

A dilute aqueous solution (1 per cent.) of strophanthin froths strongly when shaken. It is not precipitated by basic or neutral lead acetate,[1] and does not reduce Fehling's solution.

Mineral acids, as also strong acetic acid, when added to a solution of strophanthin, gradually produce a haziness. This behaviour is apparently due to the separation of strophanthidin, as it is accompanied in each case by a formation of glucose.

Solutions of caustic alkalies, ammonia, baryta, &c., turn the solution of strophanthin light yellow, but no glucose is formed at the ordinary temperature even on standing. When boiled, the alkaline liquids become deep reddish-brown, and lose much of their bitterness and physiological activity.

A solution of tannin throws down strophanthin as a copious yellowish-white precipitate, which redissolves on agitation until an excess of the reagent has been added. Other alkaloidal reagents, including the potassio-iodides of bismuth, cadmium and mercury, and mercuric, platinic, auric and ferric chlorides, produce no change in solutions of strophanthin. Phospho-molybdic acid slowly produces a bright green colour, gradually changing to greenish-blue.

In the solid state, strophanthin yields various well-marked and extremely delicate colour-reactions, which have been described in detail by T. R. Fraser (*Year-Book Pharm.*, 1889, page 463).

When solid strophanthin is moistened with strong sulphuric acid, a bright green colour is immediately produced, changing very rapidly to greenish-yellow and brownish-green, and in an hour or two becoming dirty brown without any green shade. If strophanthin moistened with strong sulphuric acid be heated to 50°–60° C., the green colour first produced soon becomes dark olive, changing to very dark brown, then to violet and dark violet-blue, and finally to black with a violet tint. With dilute sulphuric acid, strophanthin gives a nearly colourless solution, but on heating the

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gated by J. Tillie (*Jour. Anat. and Physiol.*, xxvii. 402; xxviii. 96; abst. *Pharm. Jour.*, [3], xxiv. 344). Although derived from the *strychnos* genus, its physiological action resembles that of digitalis.

Malayan arrow-poisons have also been described by E. M. Holmes (*Pharm. Jour.*, [3], xxiii. 388); L. Wray (*Kew Bulletin*, 1891, page 259); and R. Stockman (*Pharm. Jour.*, [3], xxiii. 945; xxiv. 561).

[1] The precipitate produced by lead acetate in the solution of extract of strophanthus in water is due to kombic acid, a substance which is obtained by the decomposition of its lead salt as a scaly brownish-yellow substance, soluble in water, and strongly acid in reaction. It amounts to about 1½ per cent. of the extract.

quid to about 50° it becomes various shades of green, changing violet, and in about two hours becomes violet-black.

Strong hydrochloric acid dissolves strophanthin with pale ellow colour, afterwards deepening to brownish yellow. Weaker cid (10 per cent.) produces no colour in the cold, but on heating to 50°–60° C. a yellow coloration gradually appears, rapidly changing to green and from that to a persistent blue. Dilute nitric acid (10 per cent.) dissolves strophanthin without coloration, but on heating to about 50° a violet colour appears, changing gradually to yellowish-brown, and finally to "gamboge-yellow." M. Catillon states that strophanthin from *S. kombé*, but not from other sources, gives a pale rose colour with nitric acid, becoming orange-yellow on gently warming.

H. Helbing (*Pharm. Jour.*, [3], xvii. 924) has described the ollowing reaction, by which he states that very minute traces of trophanthin may be detected:—A minute quantity of the substance is dissolved in a drop of water, and a drop of ferric chloride dded, followed by a little concentrated sulphuric acid. A eddish-brown precipitate is formed, which, either at once or after ome hours, turns to an emerald-green body which remains nchanged for a long time. M. Catillon has pointed out that his reaction distinguishes strophanthin from digitalin, which gives blue colour when treated similarly; but the difference does not eem to be constant, for T. R. Fraser states that when strophanthin is thus treated a deep yellow colour appears, changing to ink, and disappears on stirring with a glass rod. By a slightly-odified mode of operating, Fraser states that a yellow colour is irst produced, quickly followed by streaks or patches of pink or lue, the whole assuming a pale greenish-blue colour in a short ime (*Year-Book Pharm.*, 1889, page 465).

When strophanthin is subjected to the action of dilute acids it is split up into a glucose and strophanthidin. The change occurs with great facility, even in the cold, and is produced by all but the very weakest of acids. As a consequence, many of the earlier descriptions of the active principle of strophanthus (especially those of Hardy and Gallois) apply more to the decomposition-product, strophanthidin, than to strophanthin itself.

STROPHANTHIDIN may be readily prepared by acidulating a solution of strophanthin or of the alcoholic extract of strophanthus seeds in 30 parts of water with 0·3 per cent. of sulphuric acid, and heating the liquid on the water-bath for about an hour. Abundant crystals of strophanthidin are deposited on cooling. An almost equally good yield and a purer product are obtained if the same mixture be kept for five or six days at the ordinary temperature.

If the acid be increased beyond this proportion the strophanthidin is decomposed with formation of a brown amorphous substance, much less bitter than either strophanthin or strophanthidin, insoluble in acids or water, but dissolved by alkalies and alcohol.

It is remarkable that no analysis of strophanthidin appears to have been made, although, as it crystallises with great facility, it can readily be obtained pure, and its composition would go far to establish that of strophanthin.

Strophanthidin forms colourless, slender, lanceolate crystals having an intensely bitter taste. It is very slightly soluble in cold water; moderately soluble in cold rectified spirit, amylic alcohol, and chloroform, and more soluble in warm spirit. It is extracted by chloroform and by amylic alcohol from its aqueous solutions, which are neutral to litmus and do not reduce Fehling's solution either before or after boiling with dilute acids.

Strophanthidin is extremely poisonous, its toxic activity equalling that of strophanthin, for which it might perhaps be advantageously substituted, since its sparing solubility and facility of crystallisation render it easy to obtain in a state of perfect purity.[1]

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[1] Adrian and Bardet have published results respecting the constitution of strophanthin which are not in accordance with those of other observers. They state that pure strophanthin does not reduce Fehling's solution, nor even directly after being treated with an acid; but when an aqueous solution acidulated with sulphuric or hydrochloric acid was kept at 40°–50° C. for some hours the solution became turbid, and a deposit formed on the sides of the flask. The liquid then reduced Fehling's solution, and gave distinct reactions with the ordinary alkaloidal reagents. The deposit was proved to be a resin soluble in alcohol, and precipitated by water. The authors conclude that strophanthin is a glucoside which breaks up into glucose and an alkaloid which is probably the strophanthidin of other chemists. This conclusion assumes the presence of nitrogen in strophanthin and its decomposition-product, which is quite opposed to the results of the majority of observers. Adrian and Bardet also observed indications of the presence of an alkaloid in the liquid obtained by macerating crushed strophanthus seeds in water; but they believe it to be distinct from that resulting from the decomposition of strophanthin, and more probably related to, or identical with, the alkaloid described by Hardy and Gallois under the name of ineine, the existence of which body Gerrard, Elborne, and others have failed to confirm. E. Catillon (*Jour. de Pharm.*, 1888, page 281) has found that strophanthus seeds, after the removal of all the strophanthin by exhaustion with alcohol and ether, yield to boiling acidulated water a considerable quantity of a second glucoside, together with a nitrogenous principle, which appears to be the diuretic constituent in preparations of strophanthus. This substance, which

Strophanthin is a typical muscle-poison. However introduced into the body, it increases the contractile power of all striped muscles, and renders their contraction more complete and prolonged. In lethal doses, it also destroys the normal state of partial flaccidity, and causes the rigidity of contraction to become permanent, and to pass into the rigor of death. As the result of the action on muscle, the heart is early and powerfully affected by strophanthin; indeed, by regulating the dose, a very distinct influence may be produced on the heart, while the other muscles apparently remain quite unaffected.

Strophanthus thus acts primarily on the heart, finally producing paralysis of that organ, with permanence of the ventricular systole. Pulmonary respiration continues in cold-blooded animals for several minutes after the systematic heart is paralysed. Twitches occur in the striped muscles of the body, their tonicity is exaggerated, and their functional activity finally destroyed, the muscles then being hard, and shortly afterwards acid in reaction. The reflex action of the spinal cord is suspended soon after the occurrence of heart-paralysis; but the motor conductivity of the spinal column and of the nerve-trunks continues after the striped muscles of the body are paralysed.

For medicinal purposes, a single dose of 0·0003 gramme of strophanthin is said to be recommended by English physicians, though in Vienna it has been given to the amount of 0·001 to 0·002 gramme per diem.

The value of strophanthus and its preparations as cardiac remedies is well established. The pulse-disturbance frequently disappears within a few minutes, and neither digestive disturbance nor the cumulative action which frequently results from the administration of digitalis has been observed.

J. Houdas has announced the presence in digitalis of a glucoside closely allied to strophanthin and ouabain.

Glucosides of Jalap and Scammony.

Jalap, scammony, and several allied plants contain active principles of a glucosidal nature which are commonly called "resins," though they have little in common with the true resins.[1]

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is soluble in water and in 70 per cent. spirit, but less soluble in stronger alcohol, gives some alkaloidal reactions, but appears really to be an amide, and is obtained as a calcium compound. These results render it doubtful whether the preparations of strophanthus can be advantageously replaced by the pure glucoside strophanthin.

[1] J. M. Maisch has published an interesting historical account of jalap resin and jalapin (*Pharm. Jour.*, [3], xviii. 165).

Jalapurgin or **Convolvulin**, $C_{31}H_{50}O_{16}$, called also *Jalapin* and *Rhodeoretin*, is the active principle of true jalap, the root or tuber of *Ipomœa purga* or *Convolvulus Schiedeanus*. It is a colourless, brittle substance melting at 150°. It is sparingly soluble in water, but dissolves readily in alcohol and in acetic acid. In chloroform and in essential oils jalapurgin is only sparingly soluble, and it is practically insoluble in ether. It dissolves in a solution of caustic alkali and in ammonia, forming a convolvulinate of the base. In concentrated sulphuric acid jalapurgin dissolves with fine red coloration. When treated in alcoholic solution with hydrochloric acid, jalapurgin is converted into glucose and convolvulinol, $C_{13}H_{21}O_3$ (?), which is regarded as the anhydride of convolvulinolic acid.

By the action of warm dilute nitric acid, jalapurgin yields oxalic acid and ipomeic acid, the latter body being probably identical with sebacic acid.

Jalapin, $C_{34}H_{56}O_{16}$, called by Flückiger *Orizabin*, is the active principle of the root-stalks of *Ipomœa simulans* (Tampico jalap) and *I. orizabensis*. It forms an amorphous, resinous powder melting at 150°. It is only slightly dissolved by water, but is easily soluble in alcohol; as also in ether, chloroform, and essential oils. Concentrated sulphuric acid dissolves jalapin with purple or maroon-red colour, changing to brown and finally becoming black. On treatment with dilute acids in alcoholic solution, it is hydrolysed with formation of dextrose and jalapinol, $C_{32}H_{62}O_7$ or $C_{16}H_{30}O_3+\frac{1}{2}H_2O$, a body apparently having the constitution of tetrabutyl aldehyde. The oxidation-products of jalapin are similar to those of convolvulin, from which jalapin differs by $3CH_2$.[1]

Jalapin dissolves in caustic alkalies and in ammonia; on acidulating the solution jalapic acid is liberated, a body soluble in water, but only sparingly soluble in ether.

Various distinctions between convolvulin and jalapin have been described by A. F. Stevenson (abst. *Pharm Jour.*, [3], x. 644).

Scammonin forms the principal portion of the resin of scammony,[1] the dried sap of *Convolvulus scammonia*.[2] It is very similar to, and possibly identical with, jalapin, for which it is often substituted. According to Mayer, when decomposed by acids, scammonin at once yields scammonolic or jalapinolic acid, while jalapin gives jalapinol, which is said to contain H_2O more than the former body (see footnote on last page).

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[1] In 1884 (*Chem. Centralb.*, 813), Poleck and Samuelson published a full description of jalapinol. In a more recent paper (*Chem. Centralb.*, 1892, ii. 786) Poleck states that jalapinol does not exist, the products of the hydrolysis of jalapin by hydrochloric acid being dextrose and jalapinolic acid, $C_{16}H_{30}O_3$, a body crystallising in tufts of white needles, melting at 63–64° C. (see also *Jour. Chem. Soc.*, lxiv., [1], 225; lxvi., [1], 471; and lxviii. [1], 109).

According to N. Kromer, scammonin is easily soluble in ether, alcohol, chloroform, benzene, and acetic acid. It melts at 123·6° C., and has a specific rotation of $[\alpha]_D = -23°$. Its formula is stated to be $C_{88}H_{156}O_{42}$. By the action of alkalies it is broken up into the bibasic acid, scammonic acid, $C_{22}H_{44}O_{12}$. By oxidation it yields in addition carbonic, oxalic, valeric, and sebacic acids. Mineral acids split off scammonol, valeric acid, and glucose; and, by further action, a carbohydrate which reduces Fehling's solution, has a rotation of $+17·8°$, and the phenylhydrazine compound of which melts at 191°.

Turpethin, a glucoside contained in the root of *Ipomœa* or *Convolvulus turpethum*, is a body much resembling scammonin, the chief difference between them being the insolubility of the former in ether. According to Kromer (abst. *Jour. Chem. Soc.*, 1893, i. 424) turpethin melts at 147°; has the specific rotation

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[1] *Scammony* is official in the British Pharmacopœia, which requires it to be free from starch, and to yield about 75 per cent. of resin to ether, the remainder being chiefly soluble gum and a little moisture. It should not effervesce with hydrochloric acid nor yield more than 3 per cent. of ash.

Scammony has been frequently grossly adulterated; flour, chalk, sand, dirt, &c., being among the additions met with. (See *Pharm. Jour.*, [3], xxiv., 695, 706.)

C. Govaerts (*Year-Book Pharm.*, 1874, p. 241) has published the following figures showing the variation in the composition of commercial scammony :—

	Aleppo Scammony (Good).	Ordinary Scammony.	So-called Aleppo Scammony.
Resin, ...	85	20	8
Gum, ...	4	10	3
Starch, ...	0	63	75
Ash, ...	11	7	14
	100	100	100

[2] The resin from scammony root has been substituted for true scammony. The resin from the root has a very peculiar and persistent leathery odour, while the true scammony gum possesses an equally distinctive sour cheese-like smell (M. Conroy, *Pharm. Jour.*, [3], xiv. 397).

$[a]_D = -30{\cdot}14°$; and possesses the formula $C_{76}H_{128}O_{36}$. By the action of alkalies it is converted into turpethic acid, $C_{38}H_{76}O_{24}$. By the action of mineral acids, turpethol is split off, together with isobutyric acid and a sugar resembling, but not identical with, dextro-glucose. Turpethol melts at 85·8°, and appears to be the anhydride of turpetholic acid, $C_{16}H_{32}O_4$, melting at 88·4°.

According to Spirgatis (*Annalen*, cxxxix. 41) turpethin melts at 183°, and has the formula $C_{34}H_{56}O_{16}$. It yields turpethic acid, $C_{34}H_{16}O_{18}$, by boiling with baryta, and is hydrolysed by dilute mineral acids, with formation of three molecules of glucose and one of turpetholic acid, $C_{16}H_{32}O_4$.

Much confusion exists as to the origin and nomenclature of jalap-resin and its allies. By English manufacturers and wholesale druggists "jalapin" is understood to be that resin which is insoluble in ether, and therefore corresponds in its properties with jalapurgin; but in Germany the insoluble resin is called "convolvulin," the name "jalapin" being applied to the soluble resin from *Ipomœa orizabensis* or *I. simulans*. This forms about 90 per cent. of the resin of scammony and Tampico jalap,[1] and is much cheaper and less powerful than jalapurgin, which can only be obtained in quantity from true jalap (*Ipomœa purga*). (See *Pharm. Jour.*, [3], xvii. 652; xviii. 165; xxii. 888, 908, 1060, 1079; xxiii. 20.)

Commercial "jalapin" is generally prepared by extracting the powdered jalap with alcohol, adding water till the solution becomes slightly turbid, and then digesting the hot liquid with animal charcoal. After some time the tincture is filtered, evaporated to dryness, the residue washed with hot water to remove gummy and saccharine matter, and dried.

Five samples of commercial jalapin examined by C. E. Robinson (*Pharm. Jour.*, [3], xxiii. 531) gave results leading to the conclusion that they were all prepared from true jalap (*Ipomœa purga*). They lost from 6·08 to 6·84 per cent. of moisture at 100° C., and yielded from 3·79 to 5·80 per cent. of matter to ether, the whole of the residue left undissolved by ether being soluble in rectified spirit. Similar results have been recorded by E. White (*Pharm. Jour.*, [3], xvii. 652), but one sample out of eight examined consisted of the ether-soluble resin.

JALAP.—The British Pharmacopœia (1885) requires jalap tubercules,[2] when exhausted with rectified spirit, to yield "not less than

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[1] A microscopic distinction between Tampico and Vera Cruz jalaps has been pointed out by Harlant (abst. *Pharm Jour.*, [3], xvi. 917).

[2] Spurious jalap is sometimes met with (see *Pharm. Jour.*, [3], xxii. 438; xxiv. 382). Some years since, some powdered jalap, purchased from a druggist

10 per cent. of resin, of which not more than one-tenth should be soluble in ether."[1] This is described as formed of "dark brown opaque fragments, translucent at the edges, brittle, breaking with a resinous fracture, readily reduced to a pale brown powder, sweetish in odour, acrid in the throat, easily soluble in rectified spirit, insoluble in oil of turpentine. The powder yields little or nothing to warm water, and not more than 10 per cent. to ether." T. A. Ellwood (*Pharm. Jour.*, [3], xxii. 395) considers the B.P. limit of 10 per cent. of resin from the root as too low, and likely to encourage the use of an inferior drug; while the range of solubility of the resin in ether might with advantage be increased to 20 per cent. The proportion of the resin soluble in ether appears to be the reverse of the proportion of resin in the root. Thus—

	A.	B.	C.	D.	E.	F.
Resin in root, per cent., . .	10·4	15·0	13·0	16·0	14·2	12·0
Percentage of resin soluble in ether,	12·2	10·0	10·0	8·0	8·0	9·5

On the other hand, F. H. Alcock (*Pharm. Jour.*, [3], xxiii. 107) regards the B.P. limit as too high. He proposes the following method for the assay of jalap:—1 gramme of the powdered sample, free from agglutinated lumps, is treated with 20 c.c. of amylic alcohol, and the mixture shaken occasionally during a few hours. The liquid is then filtered through cotton-wool, and the residue washed twice with 5 c.c. of amylic alcohol. The solution is agitated several times with small quantities of water at about 50° C., evaporated at 100°, and weighed. By adding a small quantity of water to the contents of the dish before evaporating, it is said that the tendency of the amylic alcohol to creep up the sides of the vessel can be avoided. The product thus obtained is said to be purer, and the yield consequently less, than when the jalap is exhausted with rectified spirit. This is only to be expected, since alcohol dissolves from jalap a notable quantity of saccharine matter, in consequence of which the British Pharmacopœia directs the resinous extract to be washed

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in Sheffield, was administered to two valuable dogs, both of which died in the course of an hour. An analysis of the "jalap" by the author showed that it consisted in great part of powdered *nux vomica* seeds.

[1] According to the U.S. Pharmacopœia of 1890, "on exhausting 100 parts of jalap with alcohol, concentrating the tincture to 40 parts, and pouring it into water, a precipitate of resin should be obtained, which, when washed with water, and dried, should weigh not less than 12 parts, and of which not over 10 per cent. should be soluble in ether."

several times with hot water, before finally drying and weighing it.

In the method of assaying jalap devised by **Squibb** (*Éphemeris*, vol. iii., June 1888), the same fact is fully realised, as is evident from the following figures obtained by the analysis of samples of commercial jalap. Nos. 1 to 7 were purchased from leading wholesale druggists in New York. No. 8 was from a lot of very superior quality. Nos. 9 and 10 represent the best Mexican jalap to be found on the London market.

No.	Loss at 100° C.	Total Extract by Alcohol.	Total Resin.	Ether-soluble Resin.	Active Resin.
1	8·20 per cent.	14·80 per cent.	7·32 per cent.	0·89 per cent.	6·38 per cent.
2	8·49 ,,	16·23 ,,	9·10 ,,	0·74 ,,	8·20 ,,
3	8·00 ,,	14·40 ,,	8·72 ,,	0·86 ,,	6·50 ,,
4	8·43 ,,	19·56 ,,	7·82 ,,	1·19 ,,	6·50 ,,
5	8·42 ,,	20·28 ,,	6·51 ,,	0·95 ,,	5·50 ,,
6	...	...	8·79 ,,	0·45 ,,	8·00 ,,
7	8·40 ,,	13·62 ,,	6·19 ,,	0·80 ,,	5·34 ,,
8	6·30 ,,	31·38 ,,	18·50 ,,	1·24 ,,	17·08 ,,
9	10·00 ,,	20·96 ,,	8·42 ,,	0·71 ,,	7·60 ,,
10	9·40 ,,	19·92 ,,	6·73 ,,	0·40 ,,	6·24 ,,

R. A. Cripps (*Pharm. Jour.*, [3], xix. 422) digests 2 grammes of the sample of jalap with 50 c.c. of rectified spirit for twenty-four hours at a gentle heat. The liquid is then cooled, filtered, and 25 c.c. of the filtrate (=1 gramme of the sample) evaporated to an extract at 100°. The residue is treated in a porcelain dish with about 20 c.c. of distilled water, and warmed to about 50° C., which softens the resin, and enables the saccharine matter to be readily dissolved out by kneading the mass with a glass rod. After cooling, the liquid is filtered, and the washing of the resin repeated. The filter is washed with rectified spirit, the washings added to the resin contained in the dish, and the whole dried at 100° C.

By this method of operating, Cripps found the resin in thirty-four samples of commercial jalap to range from 5·8 to 15·6 per cent. Fourteen of the samples contained the proportion required by the British Pharmacopœia.

By a similar process, **C. E. Robinson** (*Pharm. Jour.*, [3], xxiv. 531) found the purified resin in nine specimens of commercial jalap to range from 7·57 to 17·7 per cent. On subsequently treating the purified resin with ether, from 9·0 to 27·2 per cent. of the resin was dissolved. In the cases where a considerable proportion of the resin was dissolved by ether, Robinson suggests there may have been an admixture of unofficial jalap or other adulteration.

H. Hager (abst. *Analyst*, viii. 39; *Pharm. Jour.*, [3], xiii. 264) has suggested that the specific gravity of jalap root is available as a test of quality, and that specimens having a density less than 1·140 should be rejected. Further inquiry has shown the indication afforded by the specific gravity of jalap to be fallacious.

NON-GLUCOSIDAL BITTER PRINCIPLES.

A considerable number of neutral and non-glucosidal bitter substances receive applications in medicine and as flavouring agents, &c. In many instances their chemistry has been very incompletely studied, while their feeble affinities render their available reactions few in number and wanting in precision.

The more important members of the group, *e.g.*, the aloïns, santonin, colocynthin, picrotoxin, hop-bitter, and quassiin and other hop-substitutes are considered in the sequel.

O. Bach (*Jour. pract. Chem.*, 1874, page 188; *Year-Book Pharm.*, 1874, page 293) has published some useful hints for the detection of certain bitter principles when occurring in medicine. He points out that the bitter principles of aloes, colocynth, wormwood, and gentian are soluble in water, that those of agaric and scammony (page 146) dissolve in ether, while jalap resin is insoluble in either solvent.

The scheme on next page for the detection of the above bitter principles in medicines, &c., is a tabulated form of Bach's method of procedure.

Aloes Bitters. Aloïns.

The article known in commerce as aloes or bitter aloes is the inspissated juice which has exuded or been pressed from the leaf of various species of *Aloe*.[1] It contains a number of closely allied purgative bitter principles generically called aloïns, which can be extracted from the corresponding aloes by treatment with water.

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[1] Interesting information on the preparation of aloes from the juice of the plant is given in a paper by E. M. Holmes (*Pharm. Jour.*, [3], xxiii. 233).

<table>
<tr><td colspan="7">Extract the substance or residue with alcohol, and evaporate the filtered liquid to dryness. Exhaust the dried and powdered residue with cold water, and filter.</td></tr>
<tr><td colspan="3">Residue may contain resins of agaric, scammony, and jalap. Dry, and treat with ether.</td><td colspan="4">Solution, concentrated at 100° if necessary, is cooled, treated with mercurous nitrate in excess, and rapidly filtered.</td></tr>
<tr><td rowspan="2">Residue consists of <i>jalap</i> resin, which gives with strong sulphuric acid a brown coloration, gradually becoming blood-red, and exhaling odour of <i>jalap</i>. (See also page 146.)</td><td colspan="2">Solution. Evaporate and treat residue with warm solution of sodium carbonate.</td><td colspan="2">Precipitate is washed and dissolved in warm diluted nitric acid. <i>Colocynth</i> will be indicated by yellow coloration and separation of insoluble flocks; <i>wormwood</i> gives a brown solution, and few if any flocks. Ammonia is added in excess, and the filtered liquid evaporated to dryness. The residue is treated with warm acetic acid.</td><td colspan="2">Filtrate. Add ammonia in excess, filter, treat filtrate with barium acetate, and again filter.</td></tr>
<tr><td>Residue swells to yellow mass with nitric acid. Cold sulphuric acid dissolves it with orange-red colour, changing to cherry-red, <i>scammony</i>. (See also page 147.)</td><td>Solution. On acidifying, <i>agaric</i> resin is precipitated, insoluble in nitric acid, but dissolved by sulphuric acid with orange colour, becoming brown on heating, and decolorised by nitric acid, with separation of colourless flocks.</td><td>Residue contains <i>colocynth</i>, which gives a red coloration with strong sulphuric acid and a cherry-red with Fröhde's reagent. (See also page 166.)</td><td>Solution, if evaporated to dryness, yields a residue which, in presence of <i>wormwood</i>, is yellowish - brown, and gives with Fröhde's reagent a brown coloration, changing to green, and ultimately to violet. (See also page 165.)</td><td>Precipitate. Exhaust with alcohol, evaporate filtered solution, and test portions of residue with sulphuric acid, nitric acid, and caustic alkali, all of which give yellow colorations in presence of <i>gentian</i>. (See also pages 187, 191.)</td><td>Filtrate is orange-red in presence of aloes. Evaporate to dryness, exhaust with alcohol, again evaporate, treat residue with nitric acid, and evaporate to dryness. If <i>aloes</i> were present, the picric acid formed will give a brown-red colour when boiled with caustic alkali and a reducing agent, such as potassium cyanide.</td></tr>
</table>

W. A. Tilden (*Pharm. Jour.*, [3], ii. 845; vi. 208) divides oïns into two classes, nataloïns and barbaloïns.

1. *Nataloïns* are not reddened by treatment with nitric acid, ven on heating; and do not form any definite chloro- or bromo- erivatives which yield only picric and oxalic acids as pro- lucts of the oxidation.

2. *Barbaloïns* are reddened by nitric acid, and in addition to picric and oxalic acids yield aloëtic acid, $C_7H_2N_2O_5$, and hrysammic acid, $C_{14}H_4(NO_2)_4O_4$, as products of the treat- nent. According to Tilden the barbaloïns do not form definite hloro- or bromo-derivatives.

W. A. Shenstone (*Pharm. Jour.*, [3], xiii. 461) further dis- nguishes *α-barbaloïn* from Barbadoes aloes, which is reddened the cold by nitric acid; and *β-barbaloïns* from Jafferabad, ocotrine, and Zanzibar aloes, which are not coloured in the cold nitric acid of 1·42 specific gravity, though reddened on heating. ne β-barbaloïns are, however, reddened by cold *fuming* nitric id.

The composition of the aloïns is not accurately known, though eir formula approximates to $C_{16}H_{18}O_7$, which on the whole is most probable. According to Sommaruga (*Jahresber.*, 1874, 899) socotrine aloes contains a mixture of an aloïn of the mula $C_{15}H_{16}O_7$ with another of the formula $C_{17}H_{18}O_7$. Natal ïn is said to contain $C_{25}H_{28}O_{11}$ or $C_{24}H_{26}O_{10}$.

Most discrepant statements are also recorded respecting the ubility, physiological activity, susceptibility to air and heat, and ier characters of the aloïns (see J. F. Brown, *Pharm. Jour.*, , xvii. 678). Thus, aloïn (source doubtful) is stated by various horities to dissolve in 60, in 90, and in 500 parts of water; to be insoluble, freely soluble, and soluble in 30 parts of ohol. The British Pharmacopœia of 1885 describes aloïn urce indefinite) as sparingly soluble in cold water, more so in d rectified spirit, freely soluble in hot water or alcohol, and oluble in ether.

According to the United States Pharmacopœia of 1890, aloïn m Barbadoes aloes (*i.e.*, α-barbaloïn) is soluble in about 60 rts of cold water, in 20 parts of alcohol, or in 470 parts of her. β-barbaloïn from Socotrine aloes ("socotrin") is stated be soluble in about 60 parts of water, 30 parts of absolute cohol, 380 parts of ether, or 9 parts of acetic ether.

Aloïn from Barbadoes aloes was obtained by E. Grönewold rch. *Pharm.*, [3], xxviii. 115; abst. *Jour. Chem. Soc.*, 1880, ge 639) in small, pale yellow, prismatic needles, melting at 7°. (According to Schmidt the melting-point of barbaloïn is from 142° to 145°.) The air-dried crystals obtained from a concentrated alcoholic solution are regarded by Grönewold as containing $C_{16}H_{16}O_7 + 3$ aqua or $3\frac{1}{2}$ aqua. The moist crystals readily become discoloured, especially if exposed to light. They dissolve very sparingly in cold water, but readily on boiling, the hot solution rapidly becoming brown. Barbaloïn is only slightly soluble in ether, chloroform, carbon disulphide, benzene or petroleum-spirit. Acetic acid dissolves it readily, and this solution is not affected by the air. A bromaloïn of the composition $C_{16}H_{13}Br_3O_7 + 4$ aqua is described by Grönewold, who also obtained with difficulty a triacetyl-derivative, $C_{16}H_{13}Ac_3O_7 + \frac{1}{2}$ aqua, in soft yellow needles, and a hexacetyl-derivative forming white, hard, columnar crystals. On oxidation with chromic acid mixture barbaloïn yields acetic acid, carbon dioxide, and alloxanthin, $C_{14}H_3(CH_3)(OH)_4O_2$.

Grönewold regards the *aloïn from Curaçoa aloes* as identical with that from Barbadoes aloes.

Aloïn from Natal aloes is said by Grönewold to contain $C_{24}H_{26}O_{10}$ or $C_{23}H_{23}(O.CH_3)O_9$, with a variable quantity of water of crystallisation. It forms bright yellow scales or large well-formed crystals, which soften in a capillary tube at 180° and melt with decomposition at about 210°. These characters, together with its resistance to the action of alkalies and the presence of a methoxyl-group, distinguish nataloïn from barbaloïn. On treatment with chromic acid mixture, nataloïn yields acetic acid and carbon dioxide, quinol being probably formed in addition.

In alcoholic solution, the aloïns are neutral to litmus. Their aqueous solutions are coloured green or greenish-black by ferric chloride, and are gradually precipitated by a solution of basic lead acetate.

In acid or neutral solutions the aloïns are tolerably stable, but in presence of alkalies, in which they dissolve with orange colour, they readily undergo decomposition.

The action of dilute mineral acids on the aloïns has been studied with very discordant results. According to Czumpelik and Rochleder (1861), by treating aloïn with dilute sulphuric acid, glucose and rottlerin are produced. Kossmann describes the products of the action as glucose, aloëresic acid, $C_{30}H_{32}O_{14}$, aloëretic acid, $C_{30}H_{34}O_{15}$, and aloëretin, $C_{30}H_{18}O_{11}$. Tilden, however, denies the formation of glucose. Hlasewetz states that paracoumaric acid is formed, and Rochleder and Czumpelik obtained the same body by the action of caustic potash on aloïn.

Great discrepancies occur in the recorded statements of the proportion of aloïn present in commercial aloes. No trustworthy method of determining aloïn appears to have been devised; but the following method, described by W. A. **Tilden**, is regarded by H. C. **Plenge** (*Amer. Jour. Pharm.*, Oct. 1884) as the best practicable plan of preparing aloïn on the small scale from most varieties of aloes:—25 grammes of the sample should be dissolved in boiling water, the liquid acidulated with hydrochloric acid, and allowed to cool. It is then decanted from the precipitated resinous matter, evaporated to about 50 c.c., and set aside for two weeks for crystals to form. The liquid portion is then poured off, and the crystals pressed between folds of bibulous paper. The crude aloïn thus obtained is contaminated with a considerable quantity of resin, from which it is best purified by treating it with acetic ether, with occasional agitation, till the liquid acquires a brown colour, and the yellowish colour of the crystals can be distinguished. The liquid is then quickly and carefully poured off, and the crystals dried. Treated in this manner, Barbadoes aloes, for which the method is specially adapted, gave an average of 9 per cent. of aloïn; while Curaçoa averaged 7·5, and Bonare 7 per cent. Socotrine aloes yielded 3 per cent., but on repeating the process on the same sample, no aloïn could be obtained. It was, however, isolated to the amount of 10 per cent. by digesting one part of the aloes in three parts of alcohol for twenty-four hours, and then heating the liquid over a water-bath for two hours. After cooling, the liquid was poured off from the resin, filtered, and set aside in a loosely covered dish to crystallise. The crystals of aloïn were washed with a little alcohol, and dried.

The preparation of aloïn has been investigated more recently by T. **Woodruff** (*Pharm. Jour.*, [3], xix. 773), who, as the result of numerous experiments, recommends the following process:—20 grammes of Barbadoes aloes were heated over the water-bath with about 40 c.c. of amylic alcohol, in a flask furnished with a reflux condenser. The liquid was decanted while hot into a beaker, where it solidified on cooling, and in three days formed a mass of which the major part consisted of impure crystals of aloïn. The mass was pressed and percolated with carbon disulphide to dissolve admixed resin, and the solvent then washed out with benzene. From the dried crude aloïn thus obtained a pure product was obtained by redissolving in cold water (hot water dissolves resinous matter), filtering, and allowing the aloïn to crystallise spontaneously from the filtrate.

C. A. **Serre** (*Pharm. Jour.*, [3], xxv. 840) gives the following data illustrating the quality of typical samples of commercial aloïn, which is now employed very extensively for the manufacture of pills.[1]

	Colour.	Melting-point	Resin.	Ash.
		° C.		
A. American, .	Bright pale yellow.	116	...	...
B. American, .	Brown.	140	5·8 per cent.	1·4 per cent.
C. English, . .	Greyish-yellow.	145 (softens only).	...	4·7 "
D. German, .	Deep bright yellow.	142	...	1·3 "

Serre remarks that the ash of B was white, but the ash of C and D consisted chiefly of iron, to which impurity he attributes the dull colour of sample C. He adds that the best quantitative test for resin—the precipitation of an ammonio-alcoholic solution with a large volume of ice-water—gave negative results in the cases of C and D, although the presence of resin could be detected qualitatively, and was indicated by the colour of the preparation. Serre recommends as a test for absolute freedom from resin that 1 gramme of the finely powdered aloïn should be shaken in a test-tube with 20 c.c. of water, and allowed to stand for one minute. The resultant solution should be perfectly clear. A was the only one of the above samples which stood this test. A melting-point of approximately 116° should, according to Serre, be insisted on. This condition is not consistent with recorded melting-points of pure aloïns (pages 153, 154).

COMMERCIAL ALOES vary in physical and chemical characters according to their origin. The chief varieties are those known as Barbadoes, Cape, Natal, Hepatic, Socotrine, and Zanzibar aloes.

According to the British Pharmacopœia (1885), Barbadoes aloes is the juice, when inspissated, which flows from the transversely cut leaves of *Aloe vulgaris*; imported from Barbadoes and the Dutch West Indian Islands, and known in commerce as Barbadoes and Curaçoa Aloes. Socotrine aloes is described as the similar product from *Aloe Perryi* (Baker); imported principally by way of Bombay and Zanzibar, and known in commerce as Socotrine and Zanzibar Aloes.

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[1] Some useful hints on the preparation of aloïn on a large scale have been published by C. A. Serre (*Pharm. Jour.*, [3], xxv. 839). He points out that complete combustibility and insolubility in alcohol are no indication of the absence of resin. In preparing aloïn it is usual to employ dark aloes as being of lower price, but such a practice is held by Serre to be objectionable. The selected aloes, which should be of a liver colour and clear fracture, are dissolved in water at a temperature not exceeding 40° C., and, when solution appears complete, more water is added as long as further precipitation of resin occurs. The liquid is then allowed to stand, and the bright solution concentrated *in vacuo* and set aside to crystallise. The mother-liquor is then drawn off, and the aloïn pressed and purified by suitable solvents and recrystallisation. The last traces of resin can only be removed from the aloïn by elaborate mechanical and chemical methods, impracticable on a small scale.

The following tabular statement is compiled from the characters of Barbadoes and of Socotrine Aloes given in the British Pharmacopœia :—

	BARBADOES ALOES.	SOCOTRINE ALOES.
Colour, . . .	Varies from deep reddish-brown or chocolate-brown to dark brown or almost black.	Various shades of reddish-brown, darkening by exposure to air. ("In other cases, Socotrine aloes is more or less opaque and liver-coloured, and is then known as hepatic aloes.")
Fracture, . .	Usually dull and waxy, or sometimes smooth and glassy.	Usually smooth and resinous, or rarely rough and irregular.
Thin films, . .	Translucent; orange-brown.	Transparent; orange-ruby-red (*sic*) or orange-brown.
Powder, . .	Dull olive-yellow.	Bright tawny reddish-brown.
Odour, . . .	Strong and disagreeable.	Strong and somewhat agreeable.
Taste, . . .	Bitter and nauseous.	Very bitter.

Both these varieties of aloes are described as almost entirely soluble in proof-spirit; and as exhibiting numerous crystals when moistened with rectified spirit and examined in a thin stratum under the microscope.

Barbadoes aloes of good quality is almost wholly soluble in water, but only about 50 per cent. of Socotrine aloes dissolves. The aqueous solution of aloes is coloured dark brown by alkalies, and olive-green or greenish-black by ferric chloride.

The chief application of aloes is in medicine. Aloïn is the active ingredient of certain proprietary pills.[1] Colouring matters have been manufactured from aloes, and aloes have also been employed as hop-substitutes.

According to W. Lenz, if aloes be extracted with amylic alcohol, the solution evaporated, the residue treated with nitric acid and again evaporated, and this second residue boiled with caustic potash and potassium cyanide, a blood-red coloration will be obtained. This reaction is evidently based on the formation of picric acid by the action of nitric acid on aloïn and its subsequent reduction to picramic acid by treatment with potassium cyanide.

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[1] E. M. Holmes is of opinion that aloïn is not the only active constituent of aloes.

W. Lenz also finds that useful reactions for aloes are obtained by treating an aqueous solution of the amylic alcohol extract with basic lead acetate, mercurous nitrate, tannin, and brominated potassium bromide. The extract reduces gold chloride and alkaline cupric solutions.

A test for aloes, described by Bornträger (*Zeits. Anal. Chem.*, xix. 165), which gives fairly good results with unmixed aloes, consists in extracting the substance with alcohol, filtering, shaking the evaporated filtrate with benzol, removing the benzol layer, and agitating it with ammonia, when the aqueous layer will, on standing, assume a pink or violet-red colour if aloes be present. With most kinds of aloes a very considerable time (*e.g.*, twenty-four hours) is requisite for the development of the colour; and a similar reaction is given by rhubarb and other drugs containing chrysophanic acid. In testing beer and similar liquids for aloes, Bornträger shakes at once with benzol. According to Groves (*Pharm. Jour.*, [3], ii. 1045) Bornträger's reaction is due to a tannin-like substance and not to aloïn.

Klunge (*Archiv. Pharm.*, 1883, page 363) operates on an aqueous solution of the substance to be tested for aloes. This is further diluted with water till nearly colourless, when a drop of a solution of cupric sulphate is added. An intense yellow coloration is produced, changing to greenish on standing or with excess of copper solution. On adding a solution of sodium chloride or potassium bromide and warming, the colour changes to a deep red or reddish-violet. The same change is produced in the cold if alcohol be added.[1] But all varieties of aloes do not give the red colour, and the yellow is not observable in solutions already containing a yellow colouring matter.

Fluckiger treats the substance to be tested with strong sulphuric acid, and exposes the mixture to the vapours of nitric acid, which produce, with certain kinds of aloes, a play of colours passing through green, blue, and violet to crimson. The test is useless when a mixture is to be examined for aloes.

Cripps and Dymond (*Pharm. Jour.*, [3], xv. 633) recommend the following test for the detection of aloes in pharmaceutical preparations:—one grain of the solid substance to be tested (or the residue obtained on evaporation of the liquid) is

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[1] The author has verified Klunge's reaction, which is certainly remarkable. It is difficult to explain the effect produced by sodium chloride.

Variety of Aloes.	Bornträger's Test.	Klunge's Test.	Fluckiger's Test.	Cripps' and Dymond's Test.	
				On Dilution.	On adding Ammonia.
Barbadoes, ...	Pale rose.	Deep red.	Faint blue.	Crimson.	Deep claret.
Natal, ...	Very faint pink.	Faint red.	Deep blue.	Deep crimson.	Intense brownish-red.
Curaçoa, ...	Fine rose.	Deep red.	Faint blue.	Crimson.	Intense claret.
Hepatic, ...	Faint colour in twenty-four hours.	Nil.	Nil.	Orange-red.	Claret.
Hepatic (Indian), ...	Do.	Nil.	Nil.	Orange-red.	Pale claret.
Cape, ...	Do.	Nil.	Nil.	Orange-red.	Pale claret.
Socotrine (true), ...	Pale rose.	Faint red.	Very faint blue.	Pale crimson.	Deep claret.
,, (commercial), ...	Pale rose in twenty-four hours.	Nil.	Nil.	Orange-red.	Claret.
,, ,, ...	Do.	...	...	Orange-red.	Claret.
,, ,, ...	Do.	...	...	Orange-red.	Claret.
,, (Mocha or Zanzibar), . .	Pale rose.	Red.	Faint blue.	Crimson.	Deep claret.
Aloes juice (Natal), ...	Pale brown-pink.	...	...	Crimson.	Intense brown-red.

treated in a porcelain dish, or in a glass mortar standing on white paper, with 16 drops of strong sulphuric acid, and triturated until the whole is dissolved. Four drops of nitric acid of 1·42 specific gravity are then added, and this is followed by 1 ounce of distilled water, when, in presence of aloes, a colour will be produced varying from deep orange to crimson, according to the kind of aloes employed. The result is confirmed by adding ammonia, when the colour is intensified, usually to a deep claret. The test not only allows of the detection of aloes, but gives a fair indication of the kind of aloes under examination.

Cripps and Dymond have applied the above test to a number of samples of aloes of various kinds, and have also used Bornträger's, Klunge's, and Fluckiger's tests to the same samples. The table on page 159 shows the results obtained.

Bainbridge and Morrow (*Pharm. Jour.*, [3], xx. 570) have applied certain of the above tests to other specimens of aloes, both commercial and directly prepared from the juice of the leaves of plants of known species growing at the Royal Botanic Gardens, Kew. They observed the following facts:—When sulphuric acid is mixed with Natal aloes, and the vapour of nitric acid blown over the liquid, a deep blue colour results. This reaction was not given by any other variety. True Socotrine aloes gives no reaction with nitric acid, nor with sulphuric acid and nitric acid vapours (Fluckiger's test); but every commercial specimen examined gave an evanescent crimson with nitric acid and some faint blue colour with Fluckiger's test. On addition of nitric acid to Cape aloes, a reddish colour is at first produced, but this changes in the course of five minutes to a green, which is permanent for several hours. This reaction was always obtained with Cape aloes, and was not simulated by any other variety. The only variety of Kew aloes which gave the reaction was prepared from a specimen of *Aloe Africana*. Several of the Kew specimens, when treated with bromine-water, gave a coloration varying from pale to dark purplish-red or damson; but none of the commercial specimens yielded any similar reaction. (See also E. M. Holmes, *Pharm. Jour.*, [3], xx. 562; xxi. 899; xxiii. 232.) J. Bainbridge has described the results of similar experiments on other samples of aloes of different origins (*Pharm. Jour.*, [3], xxi. 899).

Cripps and Dymond have further applied their test to various complex mixtures, with a view of ascertaining if aloes could be detected therein when present, and whether any other substances would give a similar reaction (*Pharm. Jour.*, [3], xv. 634). They found that aloes could always be detected when present, and, of the numerous pharmaceutical preparations tried, no other substance gave a similar reaction except senna, and substances, such as rhubarb, which contained chrysophanic acid. But a nearly colourless aqueous solution of aloes is not changed by ammonia, whereas with rhubarb and other substances containing chrysophanic acid a pink colour is developed. An acetic ether extract of rhubarb is coloured deep red on treatment with strong sulphuric acid, as is a similar extract from aloes. But on subsequently adding nitric acid the colour due to aloes is intensified, whereas that produced by chrysophanic acid is immediately destroyed. This behaviour allows of the detection of aloes in presence of rhubarb, &c., while the pink colour produced in an aqueous extract on addition of ammonia permits of the converse being effected.

For the determination of aloes in mixtures, H. Hager evaporates the liquid to dryness, and macerates the cooled and pulverised residue with a mixture of two volumes of chloroform, three of benzol, and one of absolute alcohol. This dissolves the resins of jalap, scammony, myrrh, senna, guaiacum, &c. The residue, which contains the aloes intact, is treated at 50° C. with alcohol of 80 per cent. The solution is evaporated to dryness in a weighed dish, and the residue treated with 12 to 15 c.c. of a 2 per cent. solution of ammonia for every gramme of residue. To the filtered liquid an excess of lead acetate is added, and a few drops of ammonia to ensure an alkaline reaction. The precipitate, which contains all the aloes, is washed with water and mixed with ammonium sulphate. The mixture is then exhausted with 80 per cent. alcohol, and the weight of aloes ascertained from that of the residue left on evaporating the filtered alcoholic solution.

For the detection of aloes in animal matters, such as fæces, J. Dietrich (*Dorpat Thesis*, 1885; abst. *Analyst*, x. 186) digests the substance with water acidulated with sulphuric acid, then macerates for twelve hours with three volumes of strong alcohol, concentrates the filtered liquid, and agitates the residue successively with petroleum-spirit and amylic alcohol. On evaporating the latter liquid, the aloïn is obtained in a state fit for the application of the ordinary tests. Dietrich treats it with nitric acid, evaporates at 100°, dissolves the residue in alcohol, and treats the deep red solution with a drop of an alcoholic solution of potassium cyanide, which produces a rose coloration in presence of aloïn. Dietrich is of opinion that on taking aloïn or aloes the greater portion is excreted with the fæces; a small portion only is absorbed and passes mostly through the kidneys, while the remainder enters the liver, and with the bile is conveyed back into the intestines.

Artemisia Bitters.

Various species of *Artemisia* contain non-glucosidal bitter principles, of which santonin is the most important. Absinthiin is a bitter principle contained in wormwood.

Santonin, $C_{15}H_{18}O_3$, is commonly obtained from the so-called "worm-seeds" (*Flores cinæ*), really consisting of the unexpanded flower-heads of *Artemisia maritima*, *A. cina*, or of closely allied varieties, which contain from 1½ to 2 per cent. of the bitter principle.[1]

Santonin is the anhydride or lactone of santoninic acid, $C_{15}H_{20}O_4$, a derivative of naphthalene. A large number of isomeric and closely allied compounds are known, as indicated by the following formulæ :—

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[1] For the preparation of santonin from worm-seeds, the volatile oil is first extracted by petroleum-spirit, and 200 grammes of the residue then boiled with 70 grammes of slaked lime, 400 c.c. of water, and 400 c.c. of rectified spirit, this treatment being twice repeated. The liquid is filtered, evaporated to about 500 c.c., and a little hydrochloric acid added. The greenish resin thereby separated is filtered off, and the filtrate treated with a slight excess of hydrochloric acid. This liberates santoninic acid, which soon decomposes with formation of santonin, which crystallises out. The product is purified by washing with cold water, solution in alcohol, decolorisation by animal charcoal, and recrystallisation.

The manufacture of santonin on a large scale is carried out at Tschimkent, which is conveniently situated to the principal source of the plant on the Kirghiz steppes. The worm-seeds are ground with lime and water, hot alcohol added to the cooled mixture, the alcohol distilled off, and the residual liquid neutralised with hydrochloric acid. The crude santonin which crystallises out after a few days is washed with cold water. During the process of extraction a quantity of resinous substance is formed, which on treatment with a warm solution of sodium carbonate yields a large quantity of santonin. The animal charcoal which is employed to decolorise the alcoholic solution absorbs a large quantity of santonin. The yield of pure santonin obtained amounts to 1·8 to 2·0 per cent. of the plant originally taken. A. Busch recommends the separation of the accompanying resin from crude santonin by the addition of lead acetate to the alcoholic solution (*Jour. prakt. Chem.*, xxxv. 322; abst. *Jour. Soc. Chem. Ind.*, 1887, 559). According to a modified process, the worm-seed is treated with milk of lime, the extract precipitated by sodium carbonate, and the filtered liquid warmed to 70° and decomposed by sulphuric acid. On cooling, santonin separates in large crystals.

To test the solutions, &c., obtained in the process of extraction for santonin, J. Kossanowski precipitates any colouring matter with basic lead acetate, and gently heats a few drops of the filtrate in a porcelain crucible. Concentrated sulphuric acid is then added, which on further heating will produce a violet coloration, the intensity of which is an indication of the amount of santonin present (*Dingl. polyt. Jour.*, cclxviii. 42; abst. *Jour. Soc. Chem. Ind.*, 1888, pp. 422, 458).

Santinic acid and Iso-santinic acid, ...	$C_{15}H_{16}O_2$
Hyposantonin, Iso-hyposantonin, and Dihydro-santinic acid and Dihydro-iso-santinic acid, . . .	$C_{15}H_{18}O_2$
Santoneous or Hyposantonic acid, and Hydrosantonide,	$C_{15}H_{20}O_3$
Santonin, and α- and β-metasantonin, ...	$C_{15}H_{18}O_3$
Santoninic acid, Santonic acid, *m*- and *p*-Santonic acids, and Photosantolactone, ...	$C_{15}H_{20}O_4$
Hydrosantonic acid, ...	$C_{15}H_{22}O_4$
Photosantonic acid, and Iso-photosantonic acid, . .	$C_{15}H_{22}O_5$

The allies and derivatives of santonin have been studied in detail by Gucci, by Grassi-Cristaldi (*Gazzetta*, xx. i. 1 ; abst. *Jour. Chem. Soc.*, 1892, p. 869), and by J. Klein (*Ber.* xxv., § 317).

Santonin crystallises in flattened columns, in feathery radiating groups, or in pearly plates having a slightly bitter taste. It has a specific gravity of 1·247 at 20°, melts at 168°–170° C., and when heated cautiously may be sublimed unchanged. When more strongly heated santonin becomes reddish brown, evolves white fumes, and on cooling sets to a clear brown vitreous mass, which is reddened on treatment with a little dry caustic alkali or slaked lime.

On exposure to light, especially to direct sun-light, santonin acquires a yellow colour. The hot alcoholic solution of this altered substance is yellow, but deposits crystals of colourless santonin on cooling.

Santonin is very sparingly soluble in cold water (1 : 5000 at 15° C.), but dissolves in 250 parts of boiling water. It is soluble in 40 parts of cold rectified spirit, and in 3 parts at the boiling-point ; in 160 parts of cold or in 42 of boiling ether, and in 4 parts of chloroform. The solution of santonin in boiling water is scarcely perceptibly bitter, but the cold alcoholic solution has an extremely bitter taste, and is lævo-rotatory. Solutions of santonin do not redden litmus, but the solid substance dissolves readily in alkaline liquids to form santoninates. On adding excess of hydrochloric acid to the alkaline solution, and immediately shaking the milky liquid with ether, santoninic acid is extracted.

Santoninic acid, $C_{15}H_{20}O_4$, rapidly separates from its ethereal solution in granules, and by recrystallisation from alcohol is obtained in fine rhombic crystals. It does not become yellow on exposure to light, and is very sparingly soluble in cold but more readily in hot water. The solution has an acid reaction, and is lævo-rotatory. When heated for some time at 120°, santoninic acid decomposes into santonin and water, and the same reaction occurs more readily on addition of a mineral acid to its solution, especially on warming.

Sodium santoninate, $C_{15}H_{19}NaO_4 + 3\frac{1}{2}$ aqua, forms colourless rhombic crystals, soluble in 3 parts of cold water, and also soluble in alcohol.

Calcium santoninate, formed in the extraction of santonin from worm-seed, crystallises in colourless silky needles, soluble in water and in dilute spirit, but almost wholly insoluble in absolute alcohol. The salt is not decomposed by carbon dioxide, but stronger acids cause a separation of santonin.

Santoninates of the heavy metals can be obtained by precipitation. When boiled with water such a santoninate is decomposed into pure santonin and the corresponding metallic oxide.

On boiling santonin for twelve hours with strong baryta-water, acidulating the liquid with hydrochloric acid, and shaking with ether, santonic acid, $C_{15}H_{20}O_4$, is extracted. This body is more stable than its isomer, santoninic acid. It forms rhombic crystals which melt at 161°–163°, is unacted on by light, and dissolves sparingly in water but readily in alcohol to form lævo-rotatory solutions.

When treated with excess of mineral acids, santonin forms santonin resin. This product is obtained most readily by heating santonin with concentrated hydrochloric acid under pressure. It is formed during the manufacture of santonin, and appears to be a mixture of products of its decomposition.

When solid santonin is agitated with a 5 per cent. solution of caustic potash in alcohol, it dissolves with transitory carmine-red coloration. The test may be modified by moistening a mixture of equal parts of slaked lime and sodium carbonate with alcohol, and adding santonin, when a fugitive purple-red coloration is developed.

According to A. Busch, glucose is a product of the action of dilute sulphuric acid on santonin.

If santonin be dissolved in slightly diluted sulphuric acid, the solution warmed on the water-bath, and a few drops of ferric chloride added, a ring of fine red colour, changing to purple, is developed round each drop of the reagent, and on continuing the heating the purple colour changes to brown. T. Salzer applies the test by treating 0·010 gramme of santonin with 1 c.c. of strong sulphuric acid and 1 c.c. of water. This mixture acquires a yellow colour, and on then adding ferric chloride and warming the violet coloration is produced.

When santonin is treated with dilute phosphoric acid, and the solution evaporated at 100°, a yellow coloration is produced, subsequently changing to purple-red.

If a solution of santonin in strong sulphuric acid be warmed on the water-bath for a few hours, and then diluted with water, iso-santonin, $C_{15}H_{18}O_3$, isomeric with santonin itself, is precipitated. When recrystallised from boiling alcohol this body melts at 138°. It also differs from santonin in being unaltered by exposure to light, and in giving no red coloration with sulphuric or phosphoric acid.

When taken internally, santonin produces a remarkable effect on the vision, all objects appearing at first of a bluish tint, but subsequently yellow or greenish yellow.[1] The taste and smell are also affected in some instances. In larger doses, santonin produces marked poisonous effects, the principal symptoms being headache, giddiness, shivering, stertorous breathing, followed by tetanus, diminished action of the heart, convulsions, and finally death by asphyxia. The pupils are dilated, and vomiting occurs in some cases. The *post-mortem* appearances are not characteristic.

In consequence of the occurrence of several accidents due to the contamination of santonin with strychnine, a test for the latter substance has been introduced into the German and United States Pharmacopœias. The latter authority prescribes the following mode of testing:—To the solution of the sample of santonin in cold concentrated sulphuric acid, water is added. The santonin will be completely precipitated, and the supernatant liquid should not have a bitter taste, nor should it be altered upon the addition of potassium bichromate (absence of brucine or strychnine), or of mercuric potassium iodide (absence of alkaloids in general).

Absinthiin, $C_{15}H_{20}O_4$, is a glucosidal bitter principle existing in wormwood, *Artemisia absinthium*, wherein it is associated with an essential oil, to which is ascribable the toxic action sometimes observed as the result of absinthe drinking. The tonic effects of wormwood are due to the absinthiin.

For the preparation of absinthiin, the plant is exhausted with ether, the ether distilled off, and the residue taken up with water. The filtered liquid is shaken with hydrated alumina, again filtered, and evaporated *in vacuo*; or shaken with ether and the ether evaporated. Absinthiin thus obtained is described by O. Senger (*Arch. Pharm.*, ccxxx. 94) as a yellowish, vitreous, intensely bitter substance, melting at 65°. It is soluble in water, alcohol, and ether. When boiled with dilute sulphuric acid, absinthiin yields dextrose, a volatile product which appears to be an ethereal oil, and an amorphous resinous compound of the formula $C_{21}H_{26}O_6$, which is apparently a hydroxy-acid of the aromatic series. It yields phloroglucol by the action of caustic alkalies, while formic, acetic, and propionic acids are among the products of its oxidation by chromic acid mixture. When treated with concentrated nitric acid, absinthiin yields oxalic and picric acids.

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[1] This curious and characteristic effect is apparently due to a direct action of santonin on the nervous elements of the retina, rendering the eye less sensitive to the rays of high refrangibility (small wave-length). The eye thus becomes colour-blind to the violet end of the spectrum. Hence only the less refrangible rays produce a visual impression, and all objects appear yellow.

Colocynth Bitter.

The fruit of the colocynth or bitter apple, *Citrullus colocynthis*, contains a neutral bitter principle called colocynthin.[1]

Colocynthin, $C_{56}H_{84}O_{23}$, (?), is extracted from bitter apples by exhausting the fruit with alcohol, evaporating the tincture to dryness, taking up the residue with water, precipitating the liquid with lead acetate, removing the excess of lead from the filtrate, and precipitating the colocynth by tannin. The compound with tannin is then decomposed by treatment with lead carbonate, and the colocynthin dissolved out by boiling alcohol.

Colocynthin is described as forming a yellowish powder or microscopic crystals. It is intensely bitter, and poisonous. Colocynthin is readily soluble in water and in boiling alcohol to form solutions neutral to litmus. The alcoholic solution is precipitated on addition of ether. Colocynthin is also insoluble in benzene and petroleum-spirit, but is soluble in acetic ether, which extracts it from its acidulated aqueous solutions. The author found it to be imperfectly extracted by chloroform, but more readily by ether.

According to Walz, colocynthin is decomposed by treatment with acids with formation of a glucose and *colocynthein*, $C_{44}H_{32}O_{13}$. G. Henke, on the other hand, states that colocynthin is unaffected by dilute acids.

E. Johannson (*Zeit. Anal. Chem.*, xxiv. 154; abst. *Jour. Chem. Soc.*, 1885, page 606) states that colocynthin, when heated with dilute sulphuric acid, yields colocyntheïn, elaterin, and bryonin. He gives various reactions of these products.

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[1] The pulp of the colocynth is official in the British Pharmacopœia, which describes it as not coloured blue by iodine, and as yielding no oil to ether. Proximate analyses of colchicum fruit have been published by L. E. Sayre (*Amer. Jour. Pharm.*, [4], lxvi. 274; abst. *Pharm. Jour.*, [3], xxiv. 1088). The botanical structure of colocynth has been described by W. Inglis Clark (*Pharm. Jour.*, [3], vii. 509), and its adulterations discussed by R. C. Tichborne, N. B. Barnes, and W. I. Clark (*Pharm. Jour.*, [3], ix. 229, 259).

Concentrated sulphuric acid dissolves colocynthin with orange-red colour, carbonisation occurring on heating. In concentrated hydrochloric acid colocynthin dissolves with coloration, and on boiling a dark green greasy substance is precipitated, which, after drying over sulphuric acid, is only partially soluble in ether. The mother-liquor from which this precipitate is obtained reduces Fehling's solution.

With sulphomolybdic acid, colocynthin is said by Johannson to give a cherry-red coloration; with sulphovanadic acid, a blood-red colour changing to blue at the edges; and a yellow coloration with alcohol and sulphuric acid (distinction from solanine and solanidine). Moistened with phenol and a drop of sulphuric acid, colocynthin gives a blood-red coloration changing to orange.

Colocyntheïn, $C_{44}H_{32}O_{13}$, is not so soluble in water as colocynthin, but is dissolved by ether and benzene, and is sparingly soluble in petroleum-spirit. It may be extracted from acidulated aqueous liquids by agitation with benzene, and the colocynthin can then be removed by ethyl acetate.

Bitters of Cocculus Indicus.

The seeds of *Anamirta paniculata* or *Cocculus Indicus* contain several bitter principles, of which picrotoxin is the most characteristic.

Picrotoxin, according to Paterno and Oglialoro, confirmed by Schmidt and Löwenhardt, contains $C_{30}H_{34}O_{13}$. For the extraction of picrotoxin, Barth and Kretschy exhaust the cocculus berries with boiling alcohol or petroleum-spirit, evaporate the filtered solution, and extract the fatty residue with water. The aqueous extract is precipitated with neutral lead acetate, filtered, the filtrate treated with sulphuretted hydrogen, and the filtered liquid concentrated to a small bulk, when crystals of impure picrotoxin separate on cooling.

Barth and Kretschy believed the product thus obtained to be a mixture of picrotoxin, picrotin, and anamirtin, which compounds they obtained from it by repeated crystallisations from benzene or water;[1] but Paterno and Oglialoro regard

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[1] Picrotin, $C_{15}H_{18}O_7$ (Paterno and Oglialoro), is prepared by the action of hydrochloric acid on the ethereal solution of picrotoxin, picrotoxinin or picrotoxide, $C_{15}H_{16}O_6$, being simultaneously formed :—

$$\underset{\text{Picrotoxin.}}{C_{30}H_{34}O_{13}} = \underset{\text{Picrotin.}}{C_{15}H_{18}O_7} + \underset{\text{Picrotoxinin.}}{C_{15}H_{16}O_6}.$$

Picrotin is also formed when a chloroformic solution of picrotoxin is allowed all three bodies as decomposition-products of true picrotoxin, and their conclusions are confirmed by the researches of Schmidt and Löwenhardt. These latter chemists point out:—(1) That true picrotoxin melts constantly at 199°–200° C.; (2) that picrotoxin does not lose weight when heated to 100°, whereas picrotoxinin loses its water of crystallisation (one molecule); (3) that picrotoxin, when treated with a large quantity of benzene in the cold, expands, whereas neither picrotin or picrotoxinin expand in the least; and (4) that picrotoxinin, so treated with benzene, undergoes no change in its composition, which fact would scarcely be the case were it a *mixture* of the two, picrotoxinin being soluble to the extent of 3·5 in 1000 of benzene, whilst the solubility of picrotin is only 0·2 in 1000 of the same menstruum.

The appearance and crystalline form of picrotoxin vary with the conditions of separation. Thus, if a fairly concentrated solution be evaporated slowly, the picrotoxin separates in well-defined prisms; but if the solution be evaporated rapidly and cooled quickly, characteristic feathery forms are deposited. When the solution is dilute, picrotoxin separates in long radiating needles. According to the United States Pharmacopœia, picrotoxin occurs as colourless, shining, prismatic crystals, or as a micro-crystalline powder.

Picrotoxin is odourless, permanent in the air, and intensely bitter. It melts at 200° C. to a yellow liquid, at a higher temperature evolves acid fumes having an odour like that of caramel, and ultimately chars.

Picrotoxin dissolves very sparingly (1 : 200) in cold water, more readily in hot (1 : 25), and is very soluble in boiling alcohol. It is also soluble in glacial acetic acid, amylic alcohol, and benzene, but is only sparingly soluble in ether or chloroform. It dissolves in ammonia and in acidulated water.

to stand in the cold, while by boiling an aqueous or benzene solution of picrotoxin that body is split up into picrotin and picrotoxinin. Picrotin crystallises with varying quantities of water. When heated it darkens at 245°, and melts at 250°–251°. It has a very bitter taste, but is not poisonous. Picrotin reduces ammonio-silver nitrate and Fehling's solution. Its solution in benzene is unaffected by boiling or by hydrochloric acid, but it is readily decomposed by alkalies. With strong sulphuric acid, picrotin develops a pale yellow colour, which changes to orange on heating.

PICROTOXININ or PICROTOXIDE, $C_{15}H_{16}O_6, H_2O$, is also formed by the action of hydrochloric acid on picrotoxin. It crystallises in rhombic plates, which become anhydrous at 100°, and melt at 200°. Picrotoxinin is bitter and very poisonous. It dissolves readily in hot water, alcohol, ether, chloroform, and benzene. It is reduced by Fehling's solution and ammonio-silver nitrate, and gives colour-reactions similar to those yielded by picrotoxin.

Anamirtin is described on page 171.

rotoxin may be extracted from acidulated aqueous liquids
itation with chloroform, ether, or amylic alcohol, the first
truum being preferable. Benzene is stated not to extract

e alcoholic solution of picrotoxin is neutral in reaction and
rotatory, but the optical activity is very variously stated.

rotoxin is neutral to litmus, but appears to possess feeble acid
rties, since it is not extracted from aqueous liquids by agita-
with immiscible solvents in presence of alkalies. It forms
llisable compounds with certain of the alkaloids.

rotoxin is not a glucoside, but it reduces Fehling's solution
ally in the cold and more rapidly on heating, the value of
ing about 20. It also reduces silver from the ammonio-
e.

rotoxin suffers ready decomposition when boiled with caustic
es.

hen a mixture of picrotoxin with an equal weight of caustic
is moistened with a drop of water, a green coloration is pro-
l, gradually changing to reddish-brown.

hen treated with cold concentrated sulphuric acid, picrotoxin
ves with bright yellow colour, darkening to orange-red on
ing, and changing very gradually to reddish-brown. The
l exhibits a brownish fluorescence.

rotoxin gives no coloration with nitric acid of 1·2 specific
y, but it dissolves in acid of 1·4 sp. grav. to a liquid which
s on evaporation a reddish-yellow residue, which becomes
t red when moistened with caustic alkali.

picrotoxin be treated with strong sulphuric acid, and a
e crystal of nitre added, the mixture gives a coloration vary-
rom red to violet on addition of excess of strong caustic

mixture of picrotoxin with cane-sugar becomes red on treat-
with strong sulphuric acid.

ueous solutions of picrotoxin are unaffected by auric. platinic,
ercuric chloride, Mayer's reagent, tannin, potassium ferro-
le and ferricyanide, and most other of the general reagents for
ids.

rotoxin is not precipitated by either neutral or basic lead
e. R. Palm has pointed out (*Jour. der Pharm.*, [5], xvii.
hat if an aqueous or alcoholic solution of picrotoxin be
n vigorously with recently-prepared and well-washed lead
xide (prepared by precipitating a solution of lead acetate
ammonia), the bitter principle is completely removed from
on. If the precipitate be separated, dried gently, and

treated with cold concentrated sulphuric acid, a bright yellow coloration will be produced, changing to reddish-violet on warming.

Picrotoxin has an intensely bitter taste, and is very poisonous. Fish appear to be specially susceptible to its action. When introduced into a very dilute solution of picrotoxin, the fish swim with uncertainty, lose their balance, and ultimately rise to the surface, lying on their sides and opening their mouths and gill-covers frequently. These symptoms, however, are by no means peculiar to poisoning by picrotoxin.[1]

A. Wynter Blyth considers frogs to be more sensitive than fish to the effects of picrotoxin. The frogs "become first uneasy and restless, and then somewhat somnolent; but after a short time tetanic convulsions set in, similar to those observed in poisoning by strychnine, but with picrotoxin an extraordinary and highly characteristic swelling of the abdomen occurs."

In toxicological inquiries, picrotoxin will be extracted when an acidulated extract of the material is shaken with chloroform or ether. From the residue left on evaporating the solvent, the picrotoxin may be dissolved out by hot water, and crystallises from the solution on concentration. Or the aqueous solution may be treated with neutral lead acetate, avoiding excess, and the filtered liquid shaken with recently prepared lead hydroxide. The precipitate may be decomposed with dilute sulphuric acid, and the picrotoxin extracted by ether, or reagents may be applied to the precipitate itself.

Cocculus Indicus, or *Anamirta paniculata*, the berries of which are the source of picrotoxin, is a small climbing shrub growing in India and the Malay Archipelago. In India the berries are employed as a drug, and picrotoxin itself is official in the United States Pharmacopœia, having replaced the powdered cocculus of earlier editions. Cocculus also forms an ingredient of an ointment having a very limited use. In England, cocculus berries were formerly employed as an adulterant of beer, but their use for this purpose is now probably entirely obsolete.[2] They have been used by fish-poachers for poisoning fish, and a preparation known as "Barlow's poisoned wheat" is stated to owe its active properties to the presence of cocculus.

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[1] A description of a "fish-poison" which is probably derived from a species of *Tephrosia* is published in the *Pharm. Jour.*, [3], xii. 885. Derrid, from *Derris eliptica*, is another body which has received a similar application (*Pharm. Jour.*, [3], xxi. 559).

[2] According to Dragendorff, who was formerly chemist to the St Petersburg police, *Cocculus indicus* has been largely used for adulterating beer in Russia, and brewers have been frequently fined for the practice, and the beer confiscated. Schubert, of Wurzburg, has stated that Bavarian beer has been often adulterated with *Cocculus indicus*. In a discussion in the House of Commons (date not recorded), Lord E. Cecil is stated to have said that the quantity of *Cocculus indicus* imported into England in 1857

From 2 to 3 grains of picrotoxin, or about 100 grains of cocculus, cause spasms and other symptoms suggestive of strychnine. Strong contraction of the uterus has been observed. Chloroform acts in antagonism to picrotoxin, and prevents the spasms caused by moderate doses.

In two recorded cases, cocculus berries have proved fatal to human beings. In 1829, several men, of whom one died, were poisoned by drinking rum containing a preparation of cocculus. In the other case, a boy aged twelve was persuaded to swallow a powder containing cocculus used for poisoning fish. He suffered intense pain throughout the whole length of the alimentary canal, followed by fever and delirium, and died on the nineteenth day. The *post-mortem* symptoms were those of peritonitis.

Cocculus Indicus berries contain from 1 to 2 per cent. of picrotoxin, together with cocculin and anamirtin (?).[1] The husks contain a non-poisonous alkaloid called menispermine,[2] but no picrotoxin.

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amounted to 68 cwt.; in 1867 the quantity had increased to 689 cwt.; and "last year," that it amounted to 1064 cwt. These amounts are not large, and may possibly have received some legitimate application, but the greater part was probably re-shipped.

[1] ANAMIRTIN is regarded by Paterno and Oglialoro as a product of the decomposition of picrotoxin, and not as a natural constituent of the cocculus berries. It remains in the mother-liquor when picrotoxin is crystallised from water. Anamirtin forms short needles, probably containing $C_{19}H_{24}O_{10}$. When heated to 280° it chars without melting. It is free from bitter taste, and is not poisonous. It dissolves in water, but is only sparingly soluble in chloroform or benzene. Anamirtin does not reduce Fehling's solution or ammonio-nitrate of silver.

COCCULIN, $C_{19}H_{26}O_{10}$, is regarded by its discoverer, Löwenhardt, as probably identical with the anamirtin of Barth and Kretschy. It crystallises in small white needles, sparingly soluble in hot water, alcohol, or ether, and insoluble in cold water or ether, but slightly soluble in cold alcohol. It is not bitter, and it does not yield the colour-reactions of picrotoxin.

[2] MENISPERMINE, $C_{18}H_{24}N_2O_2$, forms white, four-sided prisms, melting at 120°. It is insoluble in water, but dissolves in warm alcohol or ether. Menispermine has no bitter taste, and is not poisonous. It is a well-defined base with alkaline reaction.

PARAMENISPERMINE is separated from menispermine by treating the mixed bases with ether, in which the former is insoluble. It forms quadrilateral prisms or radiating crystalline masses. It melts at 250°, and at a higher temperature sublimes unchanged. The crystals are insoluble in water, and nearly so in ether, but dissolve in absolute alcohol.

Bitters and Resins of Hops.

Although the bitter principles occurring in hops are substances of considerable practical interest, and have been the subject of various investigations, their chemistry is still very imperfectly understood. The following is a *résumé* of the information on the subject.

The bitter principle of hops is stated by M. Isslieb (*Archiv. der Pharm.*, May 1880 ; *Pharm. Jour.*, [3], xi. 6) to have a composition corresponding to the formula $C_{29}H_{40}O_{10}$. He extracted it by treating the aqueous infusion of hops or " lupulin " with animal charcoal, exhausting the washed charcoal with alcohol, taking up the residue resulting from the evaporation of the alcohol with water, and shaking the solution with ether. On evaporating the ether, the bitter principle remained as a light yellow extract, which, when heated to 60°, became reddish-yellow, and could be powdered, at the same time becoming soluble with greater difficulty in cold water. It dissolved readily in alcohol, ether, and carbon disulphide, but could not be obtained crystallised. The body had a very intense pleasant bitter taste, and an aromatic odour, resembling that of hops. Alkalies dissolved the bitter with intense yellow colour. No distinct precipitate was formed by lead acetate or the general reagents for alkaloids. When the bitter was treated in warm aqueous solution with a large excess of dilute sulphuric acid (containing 5 per cent. H_2SO_4), the liquid at once became very turbid, and on standing for twelve hours deposited a brown insoluble resinous matter, lupuliretin, on the bottom and sides of the vessel, while the supernatant liquid was yellow, clear, and free from sugar. On neutralising the supernatant liquid with baryta, filtering off the precipitated barium sulphate, and evaporating the filtrate to a syrup, a crystalline barium salt was obtained of lupulinic acid. Isslieb represents the hydrolysis of hop-bitter by dilute sulphuric acid as follows :—

$$\underset{\text{Hop-bitter.}}{2C_{29}H_{46}O_{10}} + 3H_2O = \underset{\text{Lupuliretin.}}{C_{10}H_{18}O_4} + \underset{\text{Lupulinic acid.}}{C_{48}H_{82}O_{19}}.$$

Lupulinic acid, $C_{48}H_{82}O_{19}$, is described by Isslieb as crystalline, bitter, and soluble in water. It does not appear to be identical with the lupulic acid of Bungener (page 173).

Lupuliretin, $C_{10}H_{18}O_4$, is a brownish, aromatic, resinous body which could not be obtained crystalline. It is related to the essential oil and the resin of hops, and is said by Isslieb to differ from the latter by the elements of water. Isslieb suggests that hop-resin is derived from hop-oil by oxidation with elimination of the elements of water, while the substance insoluble in ether, to which Isslieb attributes the formula $C_{10}H_{18}O_6$, is perhaps a product of the simple oxidation of hop-oil. These conjectures are represented by Isslieb as follow :—

$$\underset{\text{Essential oil.}}{C_{10}H_{18}O} + O_5 = \underset{\text{Ether-insoluble body.}}{C_{10}H_{18}O_6}.$$

$$\underset{\text{Essential oil.}}{C_{10}H_{18}O} + O_4 = \underset{\text{Hop-resin.}}{C_{10}H_{14}O_3} + 2H_2O.$$

$$\underset{\text{Hop-resin.}}{C_{10}H_{14}O_3} + H_2O = \underset{\text{Lupuliretin.}}{C_{10}H_{16}O_4}.$$

The foregoing results of Isslieb are by no means in accordance with those of H. Bungener, who describes the bitter principle of hops as a crystallisable acid substance insoluble in water (*Pharm. Jour.*, [3], xiv. 1008; abst. *Jour. Chem. Soc.*, 1884, page 1366; 1886, page 809). The same substance had been previously obtained by Lermer.

LUPULIC ACID, $C_{50}H_{70}O_8$, was obtained by Bungener by exhausting the so-called "lupulin" of hops, previously freed from such impurities as hop-seeds, leaves, &c., with petroleum-ether. The solvent was distilled off in a vacuum, and the lupulic acid recrystallised without exposure to air.[1] Lupulic acid forms colourless prisms, which melt at 92°–93° and are insoluble in water. It dissolves readily in alcohol, ether, chloroform, benzene, and carbon disulphide; but is only sparingly soluble in cold petroleum-ether.

If an ethereal solution of lupulic acid be shaken with an aqueous solution of cupric acetate, the ethereal layer acquires a green colour from the formation of *cupric lupulate*, $CuC_{50}H_{68}O_8$, which is gradually deposited as a green crystalline powder, readily soluble in alcohol, more sparingly in ether, and insoluble in water. Bungener did not succeed in crystallising the lupulates of potassium, sodium, barium, or calcium. The first two salts are very soluble in water, and the last two insoluble in water but soluble in alcohol.

Lupulic acid reduces ammonio-silver nitrate. Valeric acid is the most characteristic product of its oxidation.

If lupulic acid be pulverised and exposed to the air, it rapidly becomes yellow, and the surface assumes a resinous consistency, while a pronounced odour of valeric acid and aldehydes is developed. This change occurs still more rapidly if a thin layer of an alcoholic solution of the acid be allowed to evaporate in the air. Far less crystallisable lupulic acid is obtainable from old hops than from new, a fact which is probably due to a similar oxidation. Although lupulic acid is at first insoluble in water, it yields a bitter solution on prolonged boiling, probably through change into the resinous product, which is soluble to the extent of 0·3 per cent., forming a very bitter, yellow solution, from which it is precipitated on adding sulphuric acid. Bungener considers that it is to this resinous substance, apparently identical with Hayduck's β-resin, that the bitter taste of beer is due; but in aqueous infusions of hops a considerable amount of unaltered lupulic acid is also present, dissolved in minute floating globules of oil.

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[1] The commercial production of lupulic acid is apparently the object of a patent by W. Linden, 1883, No. 3928.

According to Bungener, the resinous oxidation-product of lupulic acid has an antiseptic action on the lactic acid ferment, but the acetic acid ferment and the various species of yeast are unaffected by it.

Dreser (abst. *Pharm. Jour.*, [3], xvii. 971) found that unaltered lupulic acid in alcoholic solution acted as a powerful poison on frogs and guinea-pigs, the fatal dose in each case being about 0·025 gramme. In frogs, it produced paralysis of the central nervous system and of the heart; but in warm-blooded animals the action was in the direction of an intense acceleration of the respiration.[1] The resinous oxidation-product was practically destitute of toxic properties.

Hop-Resins.—Hayduck (*Bied. Centr.*, 1887, p. 694; *Jour. Chem. Soc.*, 1888, p. 187) has described three varieties of hop-resin, having the following characters:—*α-resin* is soft, soluble in petroleum-ether, and precipitable by alcoholic lead acetate; *β-resin* is also soft and soluble in petroleum-ether, but is not precipitated by lead; while *γ-resin* is hard and solid, insoluble in petroleum-ether, and not precipitated by lead acetate. This last body appears to be a product of the oxidation of hop-oil, while the β-resin is formed by the oxidation of Bungener's lupulic acid (page 173). Hayduck's soft resin α may be obtained by exhausting hops with ether, evaporating the ethereal solution, and treating the residue with alcohol, which leaves a white wax undissolved. On adding alcoholic lead acetate to this liquid, a yellow precipitate is formed of the lead compound of resin α. In the filtrate from this precipitate resins β and γ are found. β is soft and soluble in petroleum-ether, while γ is insoluble in that menstruum, but is dissolved by alcohol and ether. All three resins seem to be feeble acids. They have no definite solubility in water, but the solutions of α and β are intensely and disagreeably bitter, while that of the hard resin γ is slightly and agreeably bitter. According to Hayduck, the soft resins α and β are readily converted into the hard resin γ by exposure to air. α and β possess antiseptic properties of a high order, while the hard resin γ checks to some extent the rapidity of fermentation. This hard resin is present in hops in much larger proportion than either of the other two. On repeatedly extracting the mixed resins with water, the antiseptic power of the aqueous extract gradually diminishes, showing that the more soluble portions are the most powerfully antiseptic. It is to the two soft resins that the preservative power of hops appears to be due. These resins are distinctly inimical to the growth of the butyric bacteria, but do not appear to have much action on acetic bacteria or on sarcinæ.

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[1] F. Davis extracted the green strobiles of hops by ether, and obtained a mass of minute, white, acicular crystals, soluble in water (!), ether, and carbon disulphide. An aqueous solution of 3 grains of the crystals, injected hypodermically into the jugular vein of a cat, caused death in seven minutes. The animal appeared to be in no pain, but there was a peculiar twitching of the muscles (*Pharm. Jour.*, [3], xvii. 20).

The bitterness of hops is remarkable for its fugitive character, so that the palate is left clean, whereas quassia and other hop-substitutes leave a persistent bitter after-taste. The bitter taste of hops is probably due far more largely to the amorphous hop-resins than to the crystallisable lupulic acid.

Hops.

Commercial hops are the flowers of *Humulus lupulus*, a perennial climbing plant belonging to the nettle family. The flowers, which are the only part of the plant of any value, form peduncles consisting of a series of scales or bracts lying above each other so as to form a cone. The inner side of the scales and of the hard nutty fruit itself are covered thickly with a fine yellow dust, called "lupulin," which, under the microscope, is seen to consist of granules composed of glands, formed by the union of several simple cells. The glands contain hop-resin, hop-oil, bitter principles, &c.

In judging of the quality of hops it is usual to take into account the proportion of lupulin contained in the cones, the stickiness and the fineness of the aroma observed when the hops are rubbed between the palms of the hand, the appearance of the cones themselves, and the proportions of stalks, seeds, and impurities which accompany them. Unripe hops yield very little aroma, and impart a harsh flavour to beer; but they do not affect the fermentability of the wort.

F. Reinitzer (*Bied. Centr.*, xviii. 859; abst. *Jour. Chem. Soc.*, lviii. 431) gives the following method for the determination of lupulin in hops:—An unweighed portion of the hops is first sifted by Haberlandt's process, and any grains passing through the sieve are removed. This lupulin is then weighed, shaken with chloroform, and washed on to a dry filter with chloroform. It is then extracted with chloroform for an hour. The substance on the filter is allowed to dry in the air, and is then weighed. Its weight is that of the lupulin husks, which, subtracted from the original weight, gives the amount of true lupulin. A second weighed portion of the hops is then extracted with chloroform in a Soxhlet's apparatus. The residue is shaken on a sieve, the fragments of leaf being carefully removed. The lupulin is then brushed through, and the sifted portion again sifted to free it from grains. The pure lupulin husks now remaining are weighed, and from their weight, together with the figures previously obtained, the original weight of lupulin is calculated. Reinitzer shows by analysis that this method gives more concordant results than are otherwise obtainable.[1]

Lupulin is described in the British Pharmacopœia of 1885 as a granular, bright brownish-yellow powder, which, under the microscope, is seen to consist of minute, somewhat globular-top-shaped (*sic*), reticulated, translucent, shining glands. It burns readily, and has the agreeable odour and taste of hops. It should not leave more than about 15 per cent. of ash on incineration, and not more than about 30 or 40 per cent. should be insoluble in ether.

The United States Pharmacopœia of 1890 describes lupulin as leaving not more than 10 per cent. of ash on ignition; and as giving a considerable deposit (sand, &c.) when the sample is agitated with water and the mixture allowed to stand.

Four samples of commercial lupulin purchased by J. S. Ward in 1886, from houses of good repute, showed from 27 to 31 per cent. of ash, consisting chiefly of red sand, while the proportion of matter soluble in ether ranged from 39·4 to 54·2 per cent. (*Pharm. Jour.*, [3], xvi. 656).

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[1] Haberlandt gives the constituents of hop-cones as ascertained by mechanical separation as follows:—

Lupulin (hop-flour),	7·92 to 15·70 per cent.
Leaves,	69·79 ,, 78·36 ,,
Stems,	8·50 ,, 17·54 ,,
Ripe seeds,	0·02 ,, 7·80 ,,

A sample of hops from Wisconsin contained 20 per cent. of lupulin.

Lansing considers that no dependence can be placed on the mechanical methods of ascertaining the proportion of lupulin in hops.

In practice, an indication of the proportion of lupulin is obtained by rubbing the hops between the palms, the stickiness being greater the larger the amount of lupulin.

Ives found about 10 per cent. of lupulin in hops dried at 30° C. According to Payen and Chevalier, the proportion of crude lupulin is 13 per cent., about one-third of this (4 per cent.) consisting of minute particles of the cones themselves, resulting from the sifting.

E. Stockbridge (abst. *Jour. Chem. Soc.*, lviii. 657) points out that hops grown in Japan from European seeds are unsuitable for brewing, owing to the small amount of lupulin they contain. In different years the lupulin only amounted to 6 and 9 per cent., whilst European hops contain about 12 per cent. The proportion of lupulin depends largely on the prevalence of sunshine during the ripening season. When potassium sulphate was applied as a manure to neutralise the injurious effects of the rain, the proportion of lupulin was increased from 6 to 7 per cent.

Stockbridge also calls attention to the fact that, in good hops, there is only a slight variation in the ratio of matter soluble in alcohol to the substance afterwards dissolved out by water. The mean of a large number of analyses showed this ratio to be as 1:0·536. The results varied between 1:0·615 and 1:0·453. In accordance with this result, Stockbridge calculates the amount of lupulin in a sample of hops by determining the ratio borne by the matter soluble in alcohol to the matter afterwards dissolved by water, and multiplying this result by the factor 21·15 ($=\frac{11\cdot34}{\cdot536}$). This factor is based on the value 11·34 as the average amount of lupulin in good European hops.

Hops are valued by the brewer for their aroma, for their agreeable bitter taste, and for their power of precipitating albuminous matters. The aroma of hops is chiefly due to the volatile oil.[1] The bitter taste is due in part to one or more crystallisable acids (page 172), but chiefly to the resinous products of the oxidation of these bodies and of the volatile oil. The property of precipitating albumin is due to a variety of tannin (page 180).

The nature of the body which confers on hops their soporific effect is uncertain. Griessmayer isolated from hops a minute quantity of an alkaloidal body ("lupuline") having narcotic properties. It did not appear to be constantly present, as it could not be isolated from some of the finer kinds of hops. A minute quantity of choline (0·02 per cent.) was isolated from hops by

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[1] The peculiar cheesy odour of old hops is due to valeric acid, which is generally stated to be a product of the oxidation of the hop-oil; however, according to Bungener, hop-oil may be exposed to the air for days without perceptible change, the valeric acid being a product of the oxidation of the bitter principles.

Griess and Harrow, being probably a decomposition-product of lecithin. It is stated by Langer, that if beer be concentrated in a vacuum, the resultant extract possesses narcotic properties similar to those of opium. An alkaloidal substance, said to be extracted from American hops, which made its appearance in commerce some years since under the name of "hopeine," was proved to consist of an artificial mixture of morphine with another alkaloid, apparently atropine (*Pharm. Jour.*, [3], xvi. 185, 669, 687, 877, 885, 916).

Among other constituents of hops are a soluble gum; a soluble red colouring matter, which influences the colour of the beer; proteid matters; and bodies of coarse, disagreeable flavour, extracted by prolonged boiling with water.

The following analyses of Bohemian hops are by **König**:—

	From Saaz.	From Auscha.
Water,	9·90	10·61
Ethereal oil,	0·13	0·17
Matter soluble in alcohol,	20·12	20·97
,, ,, containing resin,	14·57	15·14
Matter insol. in alcohol dissolved by water; mineral,	5·42	5·10
,, ,, ,, ,, organic,	11·24	10·51
Tannic acid in aqueous extract,	2·52	3·18
Ash, free from carbon dioxide,	10·01	7·87
CO_2 in ash,	8·71	9·51

82·62

Analyses of West Prussian hops have been published by **L. Siewert** (*Bied. Centr.*, 1879, page 54; abst. *Jour. Chem. Soc.*, 1879, page 957).

An analysis of spent hops by **Kleeman** (*Bied. Centr.*, 1879, page 1050), to ascertain their value as fodder, showed:—Ash, 4·9; ether-extract, 6·16; proteids, &c., 16·27; non-nitrogenous matter, 45·07; and fibre, 27·60 per cent.

The *ash* of moisture-free hop-cones was found by **Way** and **Ogston** in 1849 to range from 5·95 to 8·05 per cent., of which from 14½ to 21½ per cent. consisted of phosphoric acid (P_2O_5), and from 12 to 25 per cent. of potash (K_2O). **J. Brand** has detected traces of boric acid in all parts (leaves, twigs, and flowers) of the hop plant. The stalks of wild hops also contain boric acid, but no trace could be detected in barley or malt.

The following data, obtained by **L. Aubry** at the experimental brewing station of Munich (abst. *Jour. Soc. Chem. Ind.*, 1894, pages 411, 1077), show the general character of commercial hops:—

Source.	Moisture.	Percentages on Dry Substance.							
		Water Extract.	Alcoholic Extract.	Benzene Extract.	Water Extract of Sample *previously* extracted by Alcohol.	Nitrogen.		Tannin.	Resin.
						Total.	Water-soluble.		
	Per Cent.								
1. Posen, ...	8·08	25·88	45·50	30·10	14·29	2·11	0·69	5·39	19·46
2. Saaz, ...	8·29	27·04	46·21	33·09	16·28	2·35	0·75	5·84	19·15
3. Spalter Stadt, ...	8·48	26·55	48·76	33·27	13·79	2·06	0·73	5·31	22·20
4. Würtemberg, ...	9·26	23·47	49·57	36·19	12·09	1·96	0·64	4·37	26·10
5. Hops of commerce, . . .	7·51	22·49	43·48	32·64	14·59	2·09	0·63	4·89	21·64
6. Wolnzach, ...	8·17	23·30	47·02	34·52	13·63	2·18	0·69	4·89	24·31
7. Altmark, ...	8·72	21·14	39·07	26·41	15·79	2·47	0·73	4·40	17·92
8. English, ...	7·63	25·76	41·39	27·45	11·15	2·75	0·81	4·43	15·62
9. Hops of commerce, . . .	8·35	22·15	42 00	30·85	14·52	2·26	0·75	4·02	19·81
10. Russian (1891), ...	9·38	28·80	49·66	31·06	...	2·46	...	5·33	...

The above samples were also examined after extraction of the resins with petroleum-ether, with results showing that only minute traces of tannin and nitrogenous bodies are dissolved by such treatment. Aubry considers the proportions of alcoholic and aqueous extracts of no value as a criterion of the value of the hops for brewing. The aqueous extract contains but little nitrogenous matter.

W. E. Porter (*Analyst*, 1878, p. 176) found the proportions of oil, resin, and bitter principle extracted from hops by ether to range in twelve samples from 8·80 to 14·98 per cent. On the proximate analysis of hops, see also G. O. Cech (abst. *Analyst*, vi. 164).

Hop-tannin.—According to Etti, the tannin of hops has the composition $C_{25}H_{24}O_{13}$, which substance co-occurs with a phlobaphen or anhydride of the probable formula $C_{50}H_{46}O_{25}$. These bodies have been studied by Hayduck and Goldiner (abst. *Jour. Soc. Chem. Ind.*, 1894, page 966). For their extraction they exhaust the hops successively with ether and with absolute alcohol, and then extract the tannin by repeatedly digesting the residue with alcohol of 70 per cent. The spirituous solution is then fractionally precipitated with a solution of lead acetate in 70 per cent. alcohol. The phlobaphen passes mainly into the brown precipitates first produced, while the tannin is concentrated in the yellow precipitates subsequently obtained. The precipitates are suspended in water, and decomposed by sulphuretted hydrogen, and the lead sulphide, which carries down with it most of the tannin or phlobaphen, is extracted with 70 per cent. alcohol. The separation of the tannin from the phlobaphen is completed by treating the products with ethyl acetate, in which hop-tannin is soluble but the phlobaphen is insoluble.

Hop-tannin thus obtained is a light brown powder, soluble in water and dilute spirit, very sparingly soluble in absolute alcohol, and insoluble in ether. A 0·2 per cent. solution in water is slightly yellow, and exhibits a green fluorescence. Ferric chloride produces an intense green coloration, but no precipitate. Hop-tannin slightly reddens blue litmus, precipitates dissolved albumin incompletely,[1] and (contrary to the statement of Etti) is precipitated by animal skin. On evaporating a solution of hop-tannin on the water-bath, it is partly converted into an insoluble phlobaphen. If sodium carbonate be added to the liquid before evaporating, the conversion into phlobaphen is nearly complete. The same conversion takes place rapidly if the dry tannin be heated to 140°. *Hop-phlobaphen* forms a reddish-brown powder, only partly soluble in boiling water or dilute alcohol, probably owing to further dehydration, and when heated to 130° becomes wholly insoluble. The yellow solution gives a dirty green precipitate with ferric chloride, and is precipitated by albumin and by animal skin. Hop-phlobaphen is soluble in solutions of alkaline carbonates with brown colour. Hop-phlobaphen precipitates albumin completely.

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[1] The compound formed is soluble in boiling water, but almost insoluble in cold water. Hence beer-wort after treatment with hops becomes turbid on cooling. Further precipitation occurs during fermentation, probably owing to formation of phlobaphen, but the finished beer retains traces of albumin.

For the determination of tannin in hops, Hayduck and Goldiner recommend Schröder's modification of Löwenthal's process, in which the aqueous extract is titrated with standard permanganate before and after precipitating the tannin by skin. The resins present in hops do not affect the results, and hence the previous extraction of the hops with ether is both unnecessary and undesirable.

The varieties of hops generally considered the best are those richest in tannin. Hayduck and Goldiner obtained the following proportions of tannin calculated on the dry hops:—Saaz, 2·91 per cent.; Spalt, 2·15 and 2·25; Neutomischel, 1·69; Wolnzach, 1·60 and 1·36; and Mainburg, 1·44 per cent. of tannin.

Determinations of the tannin in beer, by the same method, gave the following results:—English ale, 0·024 per cent.; Pilsen beer, 0·018; Munich beer, 0·012; and Berlin beer, 0·011 and 0·012 per cent. The influence of the tannin is shown in the much smaller quantity (mere traces) of soluble albumin present in strongly hopped English ale than in the much less hopped German beers; but as the compound of hop-tannin with albumin is not wholly insoluble in water, even when cold, a trace of albumin remains in the finished beer.

The essential oil is the constituent which gives to hops their characteristic aroma. It may be obtained to the amount of about 0·2 per cent. by distilling hops in a current of steam. In brewing, the greater part of the hop-oil is apt to be volatilised and lost during the operation of boiling. Hence it has been proposed to extract the oil from the hops before adding them to the wort, restoring the oil at a later stage of the manufacture. According to Personne, hop-oil is a mixture of a terpene, $C_{10}H_{16}$, with a stearoptene, $C_{10}H_{16}O$. A. C. Chapman (*Jour. Chem. Soc.*, xlvii. 54) obtained 140 c.c. of essential oil by distilling 80 kilogrammes of hops with water. After an interval of eleven months this oil yielded, on fractional distillation, about 40 c.c. of an oil consisting mainly of a sesquiterpene, $C_{15}H_{24}$, boiling between 256° and 261°. Another sample of hop-oil, similarly obtained by distilling hops with water, but examined without delay, boiled at a slightly lower temperature, and consisted of lower terpenes, sesquiterpenes, and an oxygenated constituent.

Hop-oil volatilises slowly at ordinary temperatures. It dissolves in about 700 parts of water, and is readily soluble in alcohol and ether.

In order to preserve hops they are kiln-dried. Sulphur is sometimes sprinkled on the kiln-fires with the view of preserving and bleaching the hops, which are much improved in appearance by the treatment. The practice is considered by some authorities to deteriorate the product, but is useful when the hops are attacked by mildew or parasitic growths.[1] The practice of sprinkling the growing hops with flowers of sulphur, to prevent mildew, blight, &c., is objectionable, if particles of sulphur remain on the hops after picking. Spraying the hops with sulphur and soft soap is still more liable to affect injuriously the quality of the product. The addition of powdered sulphur to a fermenting wort does not affect the activity of the yeast, but traces of sulphuretted hydrogen are invariably produced.

For the detection of sulphur in hops it is usual to exhaust 10 grammes of the sample with water, and treat the wash-water with granulated zinc and diluted hydrochloric acid, free from any trace of sulphurous acid or of free chlorine.[2] The gas evolved is passed into a dilute solution of pure caustic soda, which is subsequently tested with sodium nitroprusside or other reagent for sulphuretted hydrogen. A preferable plan is to distil the hops with steam. On separating the volatile oil from the condensed water, and treating it with an acid, preferably phosphoric acid, sulphuretted hydrogen is evolved, and can be recognised by its odour and reactions with lead acetate and sodium nitroprusside. The oil from unsulphured hops is stated to give no such reaction (G. Huhnemann, *Chem. Centr.*, 1875, p. 573). It is asserted that exhausted hops have been systematically treated with a tincture of wormwood, re-dried, and sold as a genuine article (*Analyst*, iv. 240).

Hop-Substitutes.

In seasons when hops are cheap there is little inducement for the brewer to substitute any other bitter substance for them; but in times of scarcity hop-substitutes meet with a ready sale among a certain class of brewers. *Quassia wood* is by far the most common and important constituent; but *chiretta* and other *vegetable bitters* are also employed, and some years since *picric acid* formed an ingredient of a well-known hop-substitute. It may be detected as described in Part i. page 146. *Strychnine*, many years since, was alleged to have been employed in bitter beer, but the statement has never been confirmed, and is inherently improbable. *Cocculus indicus* berries, which contain the poisonous bitter principle picrotoxin (page 167), were formerly used in beer, but there is no authentic record of such an application of late years. (See footnote on page 170.)

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[1] The advantages of sulphuring appear to be wholly on the side of the dealer (see *Wochenschr. f. Brauerei*, 1895, p. 912).

[2] According to Windisch the treatment with the reducing agent must not be prolonged beyond half an hour, or the formation of sulphuretted hydrogen from the proteid matters may lead to false conclusions.

The composition of commercial hop-substitutes is kept jealously secret, and almost the only information published in recent years on such preparations is contained in a note by W. Chattaway and the author (*Analyst*, 1887, xii. 112).[1]

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[1] In sample "A," by examination with a lens, quassia, chiretta, and hops were detected without difficulty; and there was also present a seed apparently belonging to a cruciferous plant, the exact nature of which was not ascertained.

Sample "B" consisted largely of rosin mixed with catechu or cutch, or some closely analogous tannin extract. Considerable quantities of fish-gelatin and chiretta were also present, and sodium sulphite was detected in addition. Whatever may be the value of fish-gelatin, sodium sulphite, and tannin in the manufacture of beer, they cannot be legitimately termed "hop-substitutes." The object of adding rosin is not very apparent; possibly it may have been an attempt to replace hop-resin. The proportions of the several constituents of this sample may be inferred from the following statement of the results of its treatment by solvents, &c.:—

Moisture; driven off at 100° C., ...	8·5	per cent.
Ether extract; consisting of nearly pure *colophony*, .	36·3	,,
Alcoholic extract; chiefly tannin and other constituents of *cutch*, ...	16·6	,,
Aqueous extract, ...	27·3	,,
Insoluble matter (by difference), ...	11·3	,,
	100·0	,,

Ash, ... 10·0 per cent.

Other samples of advertised hop-substitutes were examined less completely. Quassia was distinctly recognised in the decoctions of two, and other bitter substances were also present. No picric acid or calumba was present in any case. Pyrethrum ("Persian insect powder") was almost certainly a constituent of one preparation.

A curious difference was observable in the prices charged by the manufacturers of the advertised hop-substitutes. Thus the equivalent of 16 lbs. of hops was 3s., 4s., 4s., 4s. 2d., and 9d. respectively. Hence the quality and suitability of the advertised hop-substitutes for their intended purpose does not appear to be gauged by the relative amounts charged for them.

DETECTION OF HOP-SUBSTITUTES IN BEER.[1]

The detection of hop-substitutes in beer is beset with many difficulties. Beer itself is a highly complex and variable product, and some of the normal constituents add to the difficulty of detecting hop-substitutes. The problem is further complicated by the possible presence of several substitutes simultaneously, together with actual hops. The bitter principles, to which the hop-substitutes owe their employment, have in some cases been very imperfectly studied, and belong to a class of bodies not remarkable for strong chemical affinities or for characteristic reactions.[2] The most general and striking property of the majority of hop-substitutes is the intensity of their bitter taste, a character which materially increases the difficulty of detecting them, owing to the very moderate amount employed to give the beer the desired flavour.[3]

M. A. Adams (*Analyst*, xv. 123) distinguishes two classes of bitters, "fixed" and "fugitive," quassiin being a type of the former and hop-bitter of the latter. A fixed bitter affects the organ of taste in a way that is enduring, and not only does the effect of the bitter last for a long time, but it appears to overwhelm the taste-organs, so that they do not recover their entire freedom to repeat their office for a considerable period. A fugitive bitter has a far less penetrating effect, and one which lasts a comparatively short time, leaving the palate clear and free to appreciate other flavours.

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[1] This problem has at present but little practical interest, since in the United Kingdom there is no legal definition of beer, nor any standard of strength or quality, and hence the brewer has been free to employ any hop-substitute which could fairly be considered non-injurious. Bills, however, have been brought into Parliament, and on one occasion referred to a Select Committee, forbidding the unacknowledged use of hop-substitutes; and should such a provision ever become law, it will become of immediate importance to detect the more important hop-substitutes in beer, or at any rate to distinguish them from hops. The information existing on the subject in June, 1887, was collected by the author, and fully reviewed by him at the time (*Analyst*, xii. 107). A list of articles on the subject contained in English periodicals prior to that date was published in *The Analyst*, xii. 98. The results of later experiments by M. A. Adams and the author (*Analyst*, xv. 121, 180) practically complete the knowledge of the subject at the present time (February 1896).

[2] The detection of hop-substitutes in beer has formed the subject of several interesting discussions by the Society of Public Analysts. See *The Analyst*, 1887, xii. 98, 99, 103, 107, 112; xiii. 6, 43, 52; xv. 121, 135, 181.

[3] Another complication of practical importance is the very considerable quantity of beer usually recommended to be used for the analysis. Thus, if 2 litres be used for the main examination, as is recommended by Dragendorff, at least twice that quantity should be submitted to the analyst, and hence 12 litres would have to be purchased. The necessity of purchasing so large a quantity as 2½ gallons of each sample of beer would certainly render the Sale of Food and Drugs Act abortive.

Adams finds that by boiling a 2½ per cent. decoction of hops with 2½ per cent. of sulphuric acid under a reflux condenser for two or three hours every trace of bitter is destroyed, whereas quassia and its allies are not at all affected by this treatment. He suggests that the fugitive bitters are of a fermentable or glucosidal nature, and hence readily affected by dilute acids or saliva (*Analyst*, xv. 124).

One of the most valuable methods for the detection of hop-substitutes consists in treating the beer with basic lead acetate, when the hop-bitter is precipitated; and hence, if the concentrated filtrate still has a bitter taste, the presence of a hop-substitute is indicated. This distinction between the bitter of hops and those of the majority of hop-substitutes has been utilised by Dragendorff, Enders, Adams, Muter, and other chemists, so that there is a very general consensus of opinion as to its value under ordinary conditions. The author's earlier results confirmed their experience, at least when neutral lead acetate was substituted for the basic salt, a change which has the advantage that the former reagent does not precipitate certain bitter principles which are removed by the latter. With the exception of chamomile-bitter, all the bitter principles known to be or likely to be used as hop-substitutes remain in solution, while the hop-bitter is more or less perfectly precipitated, together with the whole of the phosphates, tannin, &c. Treatment of the filtrate with sulphuretted hydrogen removes the excess of lead, together with much colouring matter. The bitter principles may then be separated from the sugar, dextrin, and mineral constituents of the beer, and obtained in a state of comparative purity, by systematic treatment with immiscible solvents, as originally proposed by Dragendorff. The object being to extract as many active principles as possible in the simplest possible way, the author employs chloroform as having the most general solvent action. It separates with tolerable ease from the aqueous liquid, and should be employed as long as it leaves a notably bitter residue on evaporation. The addition of a little dilute sulphuric acid is advantageous, if not actually necessary, in some cases. The extraction with chloroform being complete, ether should next be used, the treatment being repeated as long as any notably bitter principle is extracted. Finally, the aqueous liquid is rendered alkaline with ammonia, and agitated with chloroform or ether-chloroform, to extract any alkaloids.

The following arrangement shows the behaviour of the more important bitter principles when the aqueous liquid is agitated in succession with chloroform, ether, and ammonia and ether-chloroform. The author has personally verified the behaviour of the substances to the names of which asterisks are attached.

1. Extracted by chloroform from acid solutions :—
   - Absinthiin (wormwood ; see page 165).
   - *Anthemin (chamomiles).
   - Colchicine (colchicum ; see page 3), imperfectly.
   - *Colocynthin (colocynth, or bitter apple ; see page 166), imperfectly.
   - *Calumbin, and probably some berberine (calumba), bright yellow, and highly fluorescent.
   - *Gentipicrin (gentian), very imperfectly.
   - *Picric acid (artificial), yellow, imperfectly.
   - Picrotoxin (*Cocculus indicus;* see page 167), with difficulty.
   - *Quassiin (quassia wood ; see page 187).
2. Subsequently extracted by ether from acid solutions :—
   - *Chiratin (chiretta ; page 189).
   - *Colocynthin (colocynth, or bitter apple ; page 166).
   - *Gentipicrin (gentian).
   - *Picric acid, yellow.
   - *Picrotoxin (*Cocculus indicus*).
3. Subsequently extracted by ether-chloroform from alkaline solutions :—
   - *Berberine (calumba root).
   - Colchicine (colchicum).

By evaporating off the solvent, warming the residue with a little alcohol, and then adding water, solutions are obtained which will be bitter if any of the above substances be present.[1] A very small quantity of the substance is required for this test ; indeed, the use of too large an amount must be carefully avoided, or the sense of taste will be found to be wholly paralysed for many hours subsequently.

It will be seen that chloroform or ether extracts from acidulated aqueous liquids almost the whole of the above bitters. The subsequent treatment with ether-chloroform in alkaline solution is usually unnecessary, as the principles of calumba and colchicum are in part extracted by acid chloroform.

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[1] To observe the taste of bitter principles, some of the solution should be placed on the back of the tongue with the aid of a small pipette. When there is sufficient material, some of the solution may be swallowed with advantage. The anterior part of the tongue is far less sensitive to bitter principles than the parts nearer the uvula.

The highly probable presence of a hop-substitute being indicated by the marked bitter taste of the chloroform or ether-extract, an attempt may be made to ascertain its nature; but the existing knowledge of the chemistry of the vegetable bitters available as hop-substitutes is very imperfect, and identification with absolute certainty can only be effected in the cases of a few principles, of which quassiin is the most notable and important. The following is an abstract of an extensive series of experiments made by the author in 1887 (*Analyst*, xii. 110), in which advantage was taken of all information then existing. The results of more recent researches are given on page 190.

Quassiin, $C_{32}H_{42}O_{10}$, may be obtained in a fairly pure state by exhausting quassia-wood with hot water, precipitating the solution with neutral lead acetate, removing the excess of lead from the filtrate by sulphuretted hydrogen, and shaking the filtered liquid with chloroform. On evaporation, the quassiin is obtained nearly colourless, and, with some difficulty, in a distinctly crystalline condition. Quassiin has an intensely and very persistent bitter taste. It is sparingly soluble in cold water, more readily in hot water, and is easily soluble in alcohol. Its best solvent is chloroform, which extracts quassiin readily from acidulated solutions.

An aqueous solution of quassiin does not reduce Fehling's solution, or ammonio-nitrate of silver. The solid substance gives no coloration (or merely yellow) when treated with strong sulphuric acid, or with nitric acid of 1·25 sp. gr.; nor is any colour produced on warming. These four negative reactions are important; for *picrotoxin* reduces Fehling's solution, and gives an orange-red colour with sulphuric acid; *gentipicrin* and *menyanthin* reduce ammonio-nitrate of silver, and the former gives a red colour with sulphuric acid, and the latter a yellowish-brown, changing to violet-red when warmed; and other bitters mostly give more or less characteristic reactions.

A solution of quassiin gives a white precipitate with tannin. The reaction is used by Christensen, Oliveri, and others to isolate quassiin from its solutions, and by Enders to separate it from picrotoxin. In the author's hands the reaction has not proved satisfactory. The liquid is very difficult to filter, and the filtrate still retains an intensely bitter taste, showing that the precipitation is very incomplete. As an analytical method the reaction is useless, but it is of some value as a qualitative test. The test must be made in a cold solution. Possibly a more complete precipitation of quassiin by tannic acid might be effected in an alcoholic solution.

Quassiin gives a brown coloration with ferric chloride. The reaction is best observed by moistening a quassiin residue in porcelain with a few drops of a weak alcoholic solution of ferric chloride, and applying a gentle heat. A fine mahogany-brown coloration is produced.

The most delicate and characteristic test for quassiin is based on an observation of Christensen. On treating quassiin with bromine a derivative is obtained, which is stated to be more bitter than the original substance. On adding caustic soda, the bitter taste is said to be destroyed, but a product of a fine yellow colour is obtained. The author is unable to confirm the destruction of the bitter taste, at least entirely, but the coloration is marked and characteristic. The substance to be tested for quassiin is dissolved in a little chloroform, or, if a liquid, is agitated with chloroform, and the aqueous layer separated. The chloroformic solution is then treated with bromine-water until the yellow colour remains after agitation, showing that the bromine has been used in slight excess. The aqueous liquid is then removed (or, if small in volume, may be neglected), and the chloroform agitated with ammonia. This produces immediate destruction of the colour due to the bromine, and if quassiin be absent, both the chloroform and ammoniacal liquid will be colourless. In presence of quassiin the ammonia will be coloured a bright yellow.

The author found that the chloroform-residues from camomiles, calumba, colocynth, cocculus, and chiretta did not give any similar reactions with bromine and ammonia. The *ether*-residue from chiretta gave a straw-yellow coloration gradually changing to a dull purplish-brown, but the fact that no such reaction is yielded by the chloroform extract of chiretta renders confusion with quassia impossible. Picric acid yields a solution in chloroform which is but slightly coloured compared with the deep yellow liquid produced on subsequent agitation with ammonia; but if its presence be suspected it can be readily and completely removed by agitating the chloroformic solution with soda or ammonia, and separating the alkaline liquid before employing bromine.

The author added to 1 litre of a mild beer, which had been previously proved to yield no bitter principle to chloroform after treatment with acetate of lead, sufficient infusion of quassia to make a perceptible difference in the flavour. The liquid was concentrated, precipitated with neutral lead acetate, the filtrate treated with sulphuretted hydrogen, and the refiltered liquid further concentrated and agitated with chloroform. On evaporating the chloroform a residue was obtained which had an intensely bitter taste, and yielded a solution which gave a white precipitate with tannin, but did not reduce ammonio-nitrate of silver. The residue gave no colour on warming with concentrated sulphuric acid, but gave a well-developed mahogany-brown colour with ferric chloride. By the bromine and ammonia test it gave a strong yellow coloration.

The amount of residue obtained would have sufficed to obtain all these reactions several times, so that it may be considered established that quassia can be detected with certainty and facility in a moderate quantity of beer containing it.

The employment of *chiretta* as a hop-substitute has been repeatedly recorded by previous observers, but no tests are given for it by Dragendorff or others who have worked on the subject. It was found in quantity in two hop-substitutes examined by the author, and its presence was suspected in a third. The active principle, *chiratin*, $C_{25}H_{48}O_{15}$, is intensely bitter, sparingly soluble in cold water, rather more so in hot, and is readily dissolved by alcohol and ether, the latter solvent readily removing it from its aqueous solution. On the other hand, chloroform removes but little bitter principle from an aqueous infusion of chiretta. Chiratin is a neutral substance, decomposed by dilute acids into ophelic acid and chiratogenin. It does not reduce Fehling's solution, gives a copious precipitate with tannin, and is not precipitated by neutral lead acetate. The reaction of the ether-residue from infusion of chiretta with bromine and ammonia has already been described.

Picrotoxin can be isolated and recognised by the methods described on page 169 *et seq.*

Although, in the majority of cases, treatment with neutral lead acetate removes from beer practically the whole of the hop-bitter, it is a fact that in some cases a bitter principle in marked amount remains unprecipitated, and is subsequently extracted by chloroform. As pointed out by M. A. Adams, the unprecipitable hop-bitter is specially characteristic of old hops (compare page 174).

According to J. Bell, the extractive matters of the beer interfere with the complete precipitation of the hop-bitter by lead acetate, though that reagent precipitates the bitter principles perfectly from a simple aqueous infusion of hops. Bell finds the solvent power of ether on quassia-bitter to be very limited, while the hop-bitters are readily extracted. Hence, on concentrating beer, and shaking the acidulated liquid three or four times with ether, the whole of the hop-bitter is extracted, while a large portion of the quassia-bitter is stated to remain in the aqueous liquid, and to be capable of subsequent extraction by chloroform. If the ethereal solution be evaporated, and the residue dissolved in water and treated with lead acetate, Bell finds the hop-bitter to be entirely precipitated, while the quassia-bitter remains in solution. Bell's statements are only partly confirmed by the experiments of W. Chattaway and the author. Quassiin is far too readily extracted by ether to render that solvent available for the separation of quassia from hop-bitters. On the other hand, it is generally correct that if a beer bittered with old hops be precipitated with lead acetate, the filtrate extracted by chloroform or ether, and the aqueous solution of the more or less bitter chloroform or ether-residue again treated with lead acetate, the remaining hop-bitter is in most cases entirely precipitated; but in the case of one obstinate sample of old hops, the bitter was not wholly removed even by this supplementary treatment.

To avoid the error due to imperfect removal of the hop-bitters by lead, M. A. Adams (*Analyst*, xv. 125) recommends that the infusion should be first boiled and treated with baryta-water till an alkaline solution is obtained. The brown precipitate is filtered off, and the filtrate made just acid with sulphuric acid, heated and filtered, and the filtrate evaporated to about one-fourth of its original bulk, by which time the greater part of the bitter will be destroyed. The boiling solution is next treated with basic lead acetate until precipitation is complete, when the boiling is continued for some time, and the liquid filtered. The filtrate is at once treated with dilute sulphuric acid in decided excess, the precipitate of lead sulphate filtered off, and the clear acid liquid evaporated to a small bulk at a gentle heat. It is then neutralised with chalk and filtered, when, if no bitter other than hop was present in the decoction, all bitterness will have disappeared. M. A. Adams finds that, in the case of quassia, gentian, chiretta, calumba, and many other vegetable bitters, the bitter principle is unaffected, and hence can readily be detected by the taste in the extract obtained in the above manner (*Analyst*, xv. 125).[1]

In collaboration with W. Chattaway (*Analyst*, xv. 181), the author found the foregoing method of Adams to remove entirely the bitter principle of an obstinate sample of old hops, which resisted simple precipitation by lead acetate, and even the supplementary treatment suggested by J. Bell. As the result of an extensive series of experiments, the author and W. Chattaway devised the following method of examining beer for bitter principles. Lead acetate is employed as a sorting test, but in the event of a bitter being present which is not precipitated, the filtrate is examined by the sorting action of ether and chloroform used as solvents, and the residues left by the evaporation of these menstrua are then treated with ammoniacal basic lead acetate. This reagent precipitates the bitter principles of old hops and gentian, while leaving those of quassia, calumba, and chiretta in solution. The bitter principles of these substances may be recognised to some extent by special tests. A further differentiation may also be effected by ferric acetate and other reagents. Experiments to test the process were first made on decoctions of hops, quassia, chiretta, and gentian, and as these proved successful, the process was applied to mild beer, to which sufficient of the different bittering substances was added to convert it into a fairly palatable bitter beer.

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[1] This mode of operating is not open to the objection raised against those of J. Bell and R. Bannister, described before the Select Committee on the Hop Industry in 1890. These chemists obtained beer and added quassia and other hop-substitutes in proportions largely in excess of those which would be used in practice, and thus operated under conditions unfairly favourable to their detection.

OUTLINE PROCESS FOR THE DETECTION OF BITTER PRINCIPLES IN BEER.

<table>
<tr><td colspan="6">litre of beer is evaporated to half its bulk and precipitated boiling with neutral lead acetate, the uid boiled for fifteen minutes and filtered hot. If any precipitate occur on cooling, the liquid is ain filtered.</td></tr>
<tr><td rowspan="2">ECIPITATE ntains p-bitter, ramel- tter, lelic acid om chi- tta), phos- hates, luminous atters, ., &c.</td><td colspan="5">FILTRATE. The excess of lead is removed by passing H_2S, and the filtered liquid concentrated to about 150 c.c. and tasted. If any bitter taste is perceived, the liquid is then slightly acidulated with dilute sulphuric acid, and shaken repeatedly with chloroform.</td></tr>
<tr><td colspan="2" rowspan="2">CHLOROFORM LAYER on evaporation leaves a bitter extract in the case of *gentian*, *calumba*, *quassia*, and *old hops* (only slightly or doubtfully bitter in the case of chiretta). The residue is dissolved in a little alcohol, hot water added, and the hot solution ated with ammoniacal basic lead acetate and ltered.</td><td colspan="3">AQUEOUS LIQUID is shaken with ether.</td></tr>
<tr><td colspan="2">ETHEREAL LAYER leaves a bitter residue in the case of *chiretta*, *gentian*, or *calumba*. It is dissolved in a little alcohol, hot water added, and the hot solution treated with ammoniacal basic lead acetate and filtered.</td><td rowspan="3">AQUEOUS LIQUID, if still bitter, is rendered alkaline and shaken with ether-chloroform. A bitter extract may be due to *berberine* (calumba) or *strychnine*.
The aqueous liquid, separated from the ether-chloroform, may contain *caramel-bitter* or *choline*.</td></tr>
<tr><td colspan="2">ECIPITATE contains *old hops*, *ntian*, and traces of *cara- l products*. It is suspended water, decomposed by S and the solution agi- ted with chloroform.</td><td rowspan="2">FILTRATE is boiled to remove ammonia, and treated with a slight excess of sulphuric acid, filtered and tasted. If bitter, it is agitated with chloroform, and the residue examined for *calumba* and *quassia*.</td><td rowspan="2">PRECIPITATE is treated with water and decomposed by H_2S. The filtered liquid is *bitter* in presence of *gentian*.</td><td rowspan="2">FILTRATE is treated with a slight excess of dilute sulphuric acid, filtered and tasted. A bitter taste indicates *calumba* or *chiretta*, which may be re-extracted with ether and further examined.</td></tr>
<tr><td>LOROFORM AYER is amined y special sts for ntian and d hop- itter.</td><td>AQUEOUS LIQUID contains traces of *caramel-bitter*.</td></tr>
</table>

ANIMAL BASES.

THE distinction formerly drawn between chemical individuals of the organic kingdom and those of the mineral kingdom—namely, that while the latter could be prepared artificially, the former were incapable of artificial synthesis—met its death-blow when Wöhler transformed ammonium cyanate into urea, a substance of well-defined basic properties which hitherto had been obtained solely from urine and other products of the animal kingdom. Similarly, the distinction drawn between vegetable alkaloids and animal bases is one based rather on convenience of classification and description than on any sharp difference in properties or constitution. It is a fact that the great majority of plant-bases are derivatives of pyridine or quinoline, but striking exceptions to this rule are to be found in theobromine and caffeine, which might with propriety be considered among the animal bases, since they are allied to uric acid and have the constitution respectively of dimethyl- and trimethyl-xanthine. Xanthine itself is a constituent of certain plants, though it is more characteristic of urine and urinary calculi.

Besides urea and xanthine, various other substances of basic character have been long known to exist in urine and other animal fluids, and of recent years numerous animal bases called ptomaïnes, specially characteristic of putrefactive decomposition, have been discovered, and to some extent studied.

A. Gautier has attempted to draw a distinction between those bases which are products of the action of bacteria on proteid matters and those which owe their formation to the bio-chemical and physiological activities of the living tissues. Thus, while he defined the ptomaïnes as products of bacterial fermentation or putrefaction, the leucomaïnes were regarded as resulting from normal or abnormal anaërobic decompositions of the living body. But there is no sharp distinction between the two classes of bases, and certain known animal bases are products of both kinds of decomposition. Certain of the bases of both kinds are violent poisons, equalling if not excelling in their activity the most toxic of the vegetable alkaloids, but the majority are inert, or have but a very feeble toxic action.

The animal bases of known constitution may nearly all be arranged in one or other of the following groups or classes :—

1. *Pyridine Derivatives ;* including collidine, hydrocollidine, and other bases specially characteristic of putrefactive change.

2. *Monamines ;* including methylamine, trimethylamine, and a few other similar bases.

3. *Diamines ;* including piperazine, and certain bases observed to be produced in the putrefactive decomposition of proteids.

4. *Amido-Bases ;* including glycocine, leucine, tyrosine, asparagine, &c.

5. *Betaïnes,* a special class of amido-bases ; including betaïne, choline, neurine, muscarine, &c.

6. *Urea* and its analogues and derivatives.

7. *Imido-Bases ;* including guanidine, glycocyamine, creatine, creatinine, &c. Indole and skatole may also be conveniently regarded as belonging to this class.

8. *Xanthine* or *Alloxur-Bases ;* including xanthine, hypoxanthine, guanine, carnine, &c.

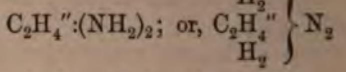
9. *Ptomaïnes.*

The most important bases of the first two classes have already been considered in Part ii. Further information respecting some of them will be found under "Ptomaïnes," as will also descriptions of numerous bases of which the constitution is not yet known. The diamines of interest which were not described in Part ii. are considered below ; the animal bases of classes 4 to 8 are described *seriatim* in the sequel ; and the ptomaïnes as a class are considered in a separate section (9).

DIAMINES.

The diamines are a class of bases derived from a double molecule of ammonia by the replacement of two or more of the hydrogen atoms by hydrocarbons of the olefine, phenylene or naphthylene series. The diamines of phenylene, toluylene, and naphthylene have been described in Part ii. (pages 86, 87, 93). Piperazine (which has the constitution of a diethylene-diamine) and its analogue spermine, are described below, while neuridine, cadaverine, and other putrefaction-products having the constitution of diamines are considered under "Ptomaïnes."

Ethylene-diamine.



$$C_2H_4'':(NH_2)_2;\ \text{or},\ \left.\begin{matrix}H_2\\C_2H_4''\\H_2\end{matrix}\right\}N_2$$

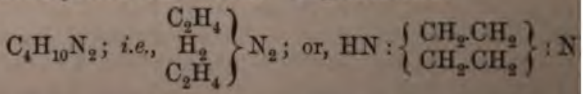
Ethylene-diamine is formed, together with several allied by the reaction of ethylene bromide and alcoholic amm 100° C. After cooling, the liquid portion is decanted fr ammonium bromide, evaporated to dryness, and distille caustic potash. The distillate is digested with solid caustic to absorb water, and the bases separated by fractional disti In the manufacture of chloral, a bye-product is obtained can be conveniently used for the preparation of ethylene-d The fraction of this product boiling between 70° and 10 tains ethylene and ethylidene chlorides, together with substitution-products. On treatment with alcoholic amm 100°–120°, the first two bodies are converted into di When the reaction is complete, the liquid is poured off fr separated ammonium chloride, and the unaltered chlorid tilled off. From the liquid left in the retort, the hydroc of ethylene-diamine separates out, and is obtained in silve needles after repeated recrystallisation and washing with al

If the brown mother-liquor be distilled with caustic and the first fraction of the distillate treated with hydr acid, a further crop of crystals of ethylene-diamine hydroc will be obtained, while the fractions subsequently distilli tain the higher diamines, triamines, &c. (Compare foot page 195.)

On distilling the hydrochloride thus prepared with potash, a hydrate of ethylene-diamine is obtained of the c tion $C_2H_4(NH_2)_2+H_2O$, from which the anhydrous base ca be obtained by repeated distillation over sodium. It is a liquid, having a faint ammoniacal odour and burning ta boils at 117°, and dissolves easily in water to a strongly liquid.

Ethylene-diamine occurs with other diamines among ducts of putrefactive decomposition of proteids (see Ptoma

Diethylene-diamine. Piperazine.



$$C_4H_{10}N_2;\ \textit{i.e.},\ \left.\begin{matrix}C_2H_4\\H_2\\C_2H_4\end{matrix}\right\}N_2;\ \text{or},\ HN:\left\{\begin{matrix}CH_2.CH_2\\CH_2.CH_2\end{matrix}\right\}:N$$

This substance has the constitution of a hexahydr zine, standing in the same relation to pyrazine (Part ii. p that piperidine does to pyridine. It may be regarded as piperidine in which the CH_2 group in the γ position has been replaced by NH. It has acquired considerable practical interest from its supposed identity with spermine,[1] a base occurring in semen and certain human organs; and from the fact of its forming a soluble urate, which character has led to its successful application as a remedy for gout and rheumatism.

Diethylene-diamine was first obtained by A. W. Hofmann, together with monoethylene-diamine, $(C_2H_4)H_4N_2$, as a product of the reaction of ammonia and ethylene bromide.[2]

Schering has patented the direct production of piperazine by reducing ethylene oxamide by zinc-dust or metallic sodium, according to the equation :—

$$\left\{\begin{matrix} CH_2.NH.CO \\ CH_2.NH.CO \end{matrix}\right\} + 4H_2 = \left\{\begin{matrix} CH_2.NH.CH_2 \\ CH_2.NH.CH_2 \end{matrix}\right\} + 2H_2O.$$

Marckwald and Haltz (*Eng. Patent*, 1892, No. 7120) have protected the manufacture of piperazine from aromatic disulphone-piperazides, a class of bodies of the following general formula, in which R represents a phenyl, tolyl, xylyl, or naphthyl residue:—

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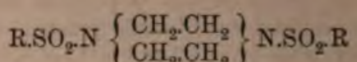
[1] The commercial products known as spermine, piperazine, and piperazidine are stated by A. W. Hofmann to be identical with diethylene-diamine, and Ladenburg admits the probable identity of the base described by him as ethylenemine with diethylene-diamine. Majert and Schmidt have come to the same conclusion (*Ber.*, xxiii. 3297, 3718, 3740). On the other hand, the natural base originally described by Schreiner under the name of spermine appears to be distinct from diethylene-diamine, and therefore the commercial synthetical product should not be called by the former name.

[2] Hofmann has shown (*Ber.*, xxiii. 3711) that on hydrolysis of the product formed by the action of ammonia on bromethylene, basic oils are obtained, which on distillation yield fractions of continually rising boiling-point, the last portions distilling above the range of a mercurial thermometer. On redistillation, the fraction passing over between 117° and 121° yields ethylene-diamine, $(C_2H_4)H_4N_2$. The fraction from 200°–225°, on addition of hydrochloric acid, gives a crystalline salt consisting chiefly of the hydrochloride of diethylene-triamine, $(C_2H_4)_2H_5N_3$. The fraction from 250°–300° gives, on addition of hydrogen bromide, triethylene-tetramine hydrobromide, $C_6H_{18}N_4.4HBr$. This salt, which is very soluble in water but only slightly so in alcohol, on treatment with caustic potash yields the free base as a colourless viscid liquid of 0·9817 specific gravity at 15°, which at 18° C. solidifies to a radiating crystalline mass melting at 12° C., and becoming limpid on gentle warming. It dissolves in water with development of much heat, forming a strongly alkaline liquid which absorbs carbon dioxide with avidity. Hofmann has also studied the action of heat on the hydrochlorides of the above bases, the most characteristic product being diethylene-diamine.



$$R.SO_2.N\left\{\begin{matrix}CH_2.CH_2\\CH_2.CH_2\end{matrix}\right\}N.SO_2.R$$

On heating the disulphone-piperazides with dilute acid to 200°–250°, or with strong sulphuric acid to 180°–200° C., they yield piperazine sulphate, from which the free base is obtained by heating with powdered caustic soda.

Piperazine was first obtained in a pure crystallised state by Majert and Schmidt, who prepared it by boiling dinitrosodiphenyl-piperazine with aqueous potash and a little alcohol.[1] It dissolves after a time, when the alcohol is distilled off, more potash added, and the product distilled until only small quantities of liquid pass over. The alkaline distillate is treated with hydrochloric acid, and the resulting piperazine hydrochloride purified by precipitation of its aqueous solution with absolute alcohol. From the hydrochloride the free base is obtained by treatment with caustic alkali and distillation.

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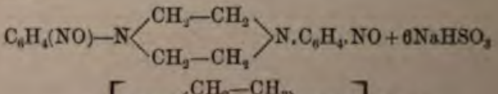
[1] This reaction has been patented by W. Majert (*Eng. Patent*, 1890, No. 15,404), who directs that 1 part of dinitrosodiphenyl-piperazine (or other analogous derivative of piperazine) should be boiled with from 2 to 4 parts of a 25 per cent. solution of caustic soda or potash, when 1 molecule of piperazine and 2 of nitrosophenol are formed. On distilling the mixture, the former passes over with the steam, and is neutralised in the distillate by hydrochloric, phosphoric, or sulphuric acid. Other modifications of the process are described in English patents, 11,957 of 1891, 4497 of 1892, and 5320 of 1893.

According to the second of these patents (B. Willcox), dinitrosodiphenyl-piperazine is converted into piperazine by means of acid sulphites according to the equation :—



The dinitrosodiphenyl-piperazine is treated with a 40 per cent. aqueous solution of the acid sulphite and the mixture heated to the boiling-point, when a violent reaction occurs, and the dinitroso-compound is completely dissolved with the formation of a yellow liquid. When the reaction is ended the solution is acidified with hydrochloric or with sulphuric acid, when a portion of the amido-phenol sulphuric acid separates, and is filtered off. The filtrate is then made alkaline and the piperazine distilled over with steam. It is purified by conversion into the hydrochloride and subsequent decomposition of the latter.

Piperazine forms well-defined, colourless, four-sided, glittering tables, which melt at 104°–107° when heated in capillary tubes; although when the melting-point is determined on larger quantities it is found to be 112°—a difference which is probably due to the extremely hygroscopic nature of the base. Piperazine, as usually met with, boils at about 140° (but at 145° when purified by treatment with sodium) and solidifies on cooling to a hard crystalline mass.

Piperazine has a faint, aromatic odour, is practically tasteless, and is neither poisonous nor caustic. It is extremely deliquescent and soluble in water, from which menstruum it crystallises in glittering quadratic tables. It is deposited from absolute alcohol in large, transparent crystals.

The aqueous solution of piperazine has a strongly alkaline reaction, while the solid substance readily absorbs carbonic anhydride from the air and is converted into the **carbonate**, melting at 162°–165°.[1]

Majert and **Schmidt** have described the following series of *hydrates* of piperazine, that most readily-formed being a **hexahydrate**, which crystallises from dilute aqueous solutions.

Hydrate.	Formula.	Melting-point, °C.
Monohydrate, ...	$C_4H_{10}N_2 + H_2O$	75
Dihydrate, ...	$C_4H_{10}N_2 + 2H_2O$	56
Trihydrate, ...	$C_4H_{10}N_2 + 3H_2O$	39–40
Tetrahydrate, ...	$C_4H_{10}N_2 + 4H_2O$	42–43
Pentahydrate, ...	$C_4H_{10}N_2 + 5H_2O$	45
Hexahydrate, ...	$C_4H_{10}N_2 + 6H_2O$	48

Piperazine hydrochloride forms snow-white matted needles, containing $B(HCl)_2 + H_2O$.[2] It is deliquescent, and very soluble in water, insoluble in alcohol, tastes like ammonium chloride, and is not poisonous. B,H_2PtCl_6 crystallises in small yellow needles, moderately soluble in hot water, but only very sparingly soluble in hot alcohol. B,H_2HgCl_4 crystallises in concentrically grouped needles, readily soluble in hot water, but reprecipitated on adding alcohol. $B,HAuCl_4$ forms small, yellow, glittering scales.

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[1] **Majert** and **Schmidt** consider that the body regarded by Ladenburg as piperazine was in reality an impure carbonate of the base, as proved by its melting-point, 159°–163°.

[2] When heated, piperazine hydrochloride is partially converted into **ethylene-imine hydrochloride**, C_2H_5N,HCl, a readily soluble salt crystallising in transparent tables. The corresponding *chloroplatinate*, B_2,H_2PtCl_6, forms small yellow prisms, while $B,HAuCl_4$ crystallises in yellow nacreous plates. The *mercurichloride* forms feathery groups of needles, while the bismuthoiodide, $3C_2H_5N,HI + 2BiI_3$, crystallises in lustrous garnet-red plates, insoluble in cold and decomposed by boiling water. On distilling this double iodide with potash, evaporating the distillate at 100°, and drying under an exsic-

Piperazine is especially characterised by the formation of an insoluble pomegranate-red double salt with bismuth, which is precipitated on adding potassium bismuth iodide (Part ii. page 138) to a dilute, slightly acid solution of piperazine hydrochloride. It forms microscopic rods or rectangular plates, insoluble in hydrochloric acid (compare spermine, page 201). By this reaction, piperazine may be directly detected in urine containing it.

Piperazine phosphate is said to be obtained as a nearly insoluble crystalline precipitate, on adding a phosphate and a little ammonia to a solution of piperazine.[1] It closely resembles spermine phosphate (page 201), but is distinguished therefrom by its crystalline form. Under the microscope it is described as appearing in small, flat, four-sided tabular crystals, the angles of which are often cut off diagonally; whereas spermine phosphate crystallises in stellate aggregates of acutely rounded pyramids (Charcot's crystals).

Piperazine urate, $C_4H_{10}N_2,C_5H_4N_4O_3$, is a salt which dissolves in 50 parts of water at 17° C. Even in presence of a large excess of uric acid, this neutral and soluble salt is formed, and is said to be capable of dissolving a large excess of uric acid.[2] The ready solubility of piperazine urate has led to the employment of piperazine hydrochloride in gout, stone and rheumatism, in doses ranging from 0·5 to 3·0 grammes daily, often in conjunction with phenacoll hydrochloride.[3] Piperazine passes through the

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cator, free ethylene-imine is obtained as a white, porcelain-like mass, which melts in sealed tubes at 159°–163°, and can be sublimed. It is readily soluble in water and alcohol, but insoluble in ether. The vapour-density (2·93) and other considerations lead Ladenburg and Abel (*Ber.*, xxi. 758) to regard the original distillate as containing $\left\{\begin{matrix}\mathrm{CH}\\\mathrm{CH}\end{matrix}\right\}\mathrm{NH}$, and the solid product as having the doubled formula $\left\{\begin{matrix}\mathrm{CH.NH.CH}\\\mathrm{CH.NH.CH}\end{matrix}\right\}$; in which case it is isomeric with piperazine (diethylene-diamine).

[1] Several samples of commercial piperazine have failed to yield any precipitate of phosphate in the author's hands.

[2] How the recorders of this observation distinguished a mixture of free uric acid with the neutral urate from a soluble acid urate is not evident. What is meant is probably that excess of uric acid does not lead to the formation of an insoluble salt.

[3] G. Roe has pointed out that when dispensed in strong solution

human organism unchanged, and may be found in the urine in a very short time.[1] It is best detected by boiling the urine, filtering from albumin or other precipitate, acidulating the filtrate with hydrochloric acid, concentrating it to a small bulk, and again filtering. The filtrate is treated with strong soda solution and distilled. In the distillate the piperazine may be recognised by adding potassio-bismuth iodide, when the characteristic bismuth compound is precipitated in garnet-red microscopic crystals.

Piperazine picrate is precipitated, on adding excess of picric acid to a solution of piperazine, in pale yellow needles, which are insoluble in cold water or in hydrochloric acid and exhibit a characteristic microscopic appearance. The salt is soluble in hot water or in a solution of piperazine.

Biesenthal found in two cases that the addition of picric acid to the urine of persons who had taken piperazine produced precipitates which he at first regarded as due to albumin; but he subsequently concluded that they were produced by piperazine itself, which passes unchanged through the system, and gives a precipitate with picric acid even when dissolved in 20,000 parts of water. Piperazine can thus be detected in the urine three or four hours after it has been taken. The picrate has a characteristic crystalline form, and cannot be mistaken for the amorphous precipitate produced by albumin. Further, the precipitate of piperazine picrate dissolves on heating, and reappears on cooling, while that due to albumin is permanent. The nature of the precipitate may be proved by treating it with hydrochloric acid, removing the picric acid by agitation with ether, and recognising the piperazine by its reaction with potassio-iodide of bismuth.

Piperazine is incompatible with phenacoll hydrochloride, and E. H. Gane has shown that the latter compound is decomposed with formation of piperazine hydrochloride and precipitation of free phenacoll in a crystalline state. For a similar reason piperazine is incompatible with iron salts and with alkaloids.

[1] Finzelberg has pointed out that in order to ensure the full exercise of the power of piperazine to dissolve uric acid, its retention in the blood for some time should be aimed at. Hence the base is preferably given in solution, and not in pills or powders. As piperazine does not produce irritation of the mucous membrane of the bladder, even in 3 to 5 per cent. solution, it may be injected locally for breaking down urinary calculi of mixed composition. J. Fawcett finds that an aqueous solution of piperazine dissolves uric acid calculi, but a solution of it in urine of the strength of 1 in 1000, which is above that usually found in the urine after taking the drug internally, has no effect whatever (*Brit. Med. Jour.*, 1894, ii. 1426). Hirsch found that the application of piperazine in 1 per cent. solution to open gouty sores relieved the pain and reduced the inflammation (*Pharm. Centralb.*, xxxiii. 145).

Dinitroso-piperazine, $C_4H_8N_2(NO)_2$, is obtained when sod nitrite is added to a solution of piperazine hydrochloride cont ing free hydrochloric acid, and the mixture warmed for a s time. A crystalline body separates out, which, when purifie crystallisation from boiling water, forms yellowish lustrous pl melting at 158° and sparingly soluble in cold water or ether, readily in boiling water or hot ether. Dinitroso-piperazine is decomposed by boiling with caustic alkalies, or by cold centrated hydrochloric or sulphuric acid. It gives a deep coloration, after some minutes, with a solution of phenol in centrated sulphuric acid (Liebermann's reagent).

ETHYLENE-ETHENYL-DIAMINE[1] has been proposed, under name of lysidine, as a substitute for piperazine in uric diathesis. Lysidine is a very hygroscopic, reddish-white, cry line substance, having a peculiar odour resembling coniine. easily soluble, and occurs commercially as a 50 per cent. aqu solution, from 2 to 10 grammes of which are directed to be t at a time in aërated water.

Spermine is a base occurring as a crystalline phosph in the secretions and certain organs of animals. This phosp sometimes known as "Charcot's crystals," appears t especially plentiful in the spleen, liver, and blood of men animals suffering from leucocythæmia,[2] as also in the expec tions in cases of bronchial asthma. They are also found o surface of the spirit used for preserving pathological preparat and have been mistaken for tyrosine, calcium phosph and other compounds. Later researches have shown that crystals occur in various healthy tissues, but are most acteristic of semen, of which they form about 5 per cent. of solid constituents. They were prepared by P. Schrei (*Liebig's Annalen*, cxciv. 68) from fresh human semen by bo it with alcohol, separating and drying the precipitate, treati with warm water rendered alkaline with ammonia, and concen ing the filtered solution. The crystals which separate are pu by recrystallisation from hot water containing a little amm From the spermine phosphate thus prepared the free

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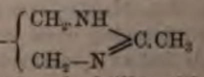
[1] This substance has the formula :—



$$\left\{\begin{matrix} CH_2.NH \\ CH_2-N \end{matrix}\right\rangle C.CH_3$$

[2] Spermine is extracted from these sources by boiling with water conta acetic acid, precipitating the solution with lead acetate, removing the of lead by sulphuretted hydrogen, and precipitating the base by pho tungstic acid. Free spermine is obtained by boiling the precipitate baryta-water, and evaporating the filtered solution.

is obtained by boiling with baryta-water, and evaporating the filtered solution.

Spermine is a colourless, odourless, crystalline substance, soluble in water and alcohol to form strongly alkaline solutions.

Spermine phosphate is immediately precipitated in crystals on adding phosphoric acid to an alcoholic or aqueous solution of free spermine. This reaction distinguishes true spermine from commercial piperazine. The crystals, which are chiefly prisms and stellate aggregates of acutely rounded pyramids, have a characteristic microscopic appearance. They are colourless, brittle, slightly soluble in cold but readily in hot water, and in dilute acid and alkaline liquids; but are insoluble in alcohol, ether, or chloroform. Spermine phosphate contains two atoms of nitrogen to one of phosphorus, loses 3 aqua at 100°, melts at 170°, and at a higher temperature decomposes with evolution of ammonia.

Spermine hydrochloride is crystalline; the chloroplatinate forms large prismatic crystals. Auric chloride precipitates from solutions of spermine hydrochloride the *aurichloride*, which crystallises in golden-yellow plates, soluble in ether, alcohol, and water. When an aqueous solution of this salt is treated with metallic magnesium, an odour resembling that of fresh human semen is evolved.[1]

Spermine bismutho-iodide crystallises long, pointed needles, often united to form feathery aggregates. The microscopic appearance of this salt affords one of the few tangible distinctions between spermine and piperazine (page 198).

The formula attributed to spermine by Schreiner was C_2H_5N, while Ladenburg and Abel suggested that it was identical with diethylene-imide, $C_4H_{10}N_2$, which has the same percentage composition. This again is very probably the same substance as piperazine. Pöhl, however, has prepared spermine by a method similar to that of Schreiner (page 200), and finds that its properties fully agree with his description; but, on the other hand, the analysis of the chloroplatinate gave numbers which did not agree with the formula C_2H_5N, but with the composition $C_{10}H_{26}N_4$, and the analysis of the aurichloride confirmed this result. Hence, if these results be correct, spermine can be neither identical with nor an isomer of piperazine.

A. Jürgens (*Chem. Central.*, 1891, i. 193; *Pharm. Zeit. Russ.*, xxix. 726) has compared Schreiner's base with that of Pöhl. He confirms the former chemist's results, and states that certain of

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[1] A. Jürgens considers that this reaction has only a negative value, since the filtrate from spermine phosphate gave the same smell when similarly treated; but it is possible that the trace of spermine remaining in solution sufficed to produce the odour.

Pöhl's specimens contained no spermine, since ammonium phosphate and ammonia gave no crystalline precipitate, auric chloride precipitated an amorphous substance, and platinic chloride gave cubical, not prismatic, crystals.

A direct comparison by **Majert** and **Schmidt** of piperazine with Schreiner's spermine has shown that these two compounds are not identical, great differences existing between the phosphates and bismutho-iodides of the two bases (*Ber.*, xxiv. 241).

AMIDES.

By the replacement of the hydrogen of ammonia by acid-radicals, or, otherwise expressed, by the replacement of the hydroxyl-group by amidogen, NH_2, amides are produced which may be primary, secondary, or tertiary, according to the number of hydrogen-atoms or hydroxyl-groups thus substituted. Thus there may be obtained from acetic acid, $C_2H_3O.OH$:—

Acetamide, ...	$(C_2H_3O).NH_2$
Diacetamide, ...	$(C_2H_3O)_2.NH$
Triacetamide, ...	$(C_2H_3O)_3.N$

Alkylated amides are compounds derived from ammonia by the simultaneous replacement of its hydrogen by acid and alkyl-radicals, as in ethyl-acetamide or acetyl-ethylamine :—

$$NH\begin{cases} C_2H_5 \\ C_2H_3O \end{cases}$$

Acetanilide (Part ii. page 68), $C_6H_5.NH.C_2H_3O$, is a body of the same class.

The amides are obtained :—

1. By heating the ammonium salts of the corresponding acids to about 230° C. :—$C_2H_3O.ONH_4 = H_2O + C_2H_3O.NH_2$.

2. By the addition of the elements of water to the cyanide of the next lower alkyl-radical :—$CH_3.CN + H_2O = CH_3.CO.NH_2$.

This assimilation of water is frequently effected by dissolving the nitrile in concentrated sulphuric acid, or in a mixture of acetic and sulphuric acids; or by the action of cold concentrated hydrochloric acid. Also, and often quantitatively, by treatment with hydrogen peroxide.

3. By the action of ammonia on acid chlorides or anhydrides :—

a. $CH_3CO.Cl + 2NH_3 = CH_3CO.NH_2 + NH_4Cl$.

b. $[(CH_3CO)O]_2 + 2NH_3 = CH_3CO.NH_2 + C_2H_3O.ONH_4$.

4. By the reaction of ethereal salts with ammonia, the change etimes occurring in the cold :—

$$CH_3CO.OC_2H_5 + NH_3 = CH_3CO.NH_2 + H.OC_2H_5 .$$

5. The secondary and tertiary amides result from the treatment the acids or anhydrides with their nitriles :—

$$a.\ CH_3.CN + CH_3.CO.OH = (CH_3CO)_2NH .$$
$$b.\ CH_3.CN + (CH_3CO)_2O = (CH_3CO)_3N .$$

foregoing reactions are those of acetamide, which is the ical body of the series.

he amides are readily saponifiable. When they contain both and alkyl-radicals only the former is saponified :—

$$\underset{\text{Ethyl-acetamide.}}{C_2H_3O.NH(C_2H_5)} + H.ONa = \underset{\text{Sodium acetate.}}{C_2H_3O.ONa} + \underset{\text{Ethylamine.}}{H.NH(C_2H_5)} .$$

itrous acid acts on the amides with formation of the corre- ding acids and liberation of nitrogen. The reaction consists n exchange of a hydroxyl-group for an amidogen-group (OH NH$_2$) :—

$$C_2H_3O.NH_2 + NO.OH = C_2H_3O.OH + H_2O + N_2 .$$

his reaction may be applied to the determination of amido- pounds (see Asparagine, page 220).

Vhen treated with bromine and alkali the primary amides form no-derivatives, which are converted by further treatment with li into amines thus :—

$$C_2H_3O.NH_2 + Br_2 = C_2H_3O.NHBr + HBr .$$

se bromo-derivatives react with more amide and alkali to form liar substituted ureas, *e.g.*, methyl-acetyl-urea, NH(CH$_3$).CO. (C$_2$H$_3$O), which are split up by further alkali with formation mines containing one carbon-atom less than the original amide. s reaction affords an excellent means of preparing the lower nes (see Methylamine, Part ii. page 11) ; but in the case of des containing more than five carbon-atoms the reaction is productive of amines, as it is complicated by the formation nitriles and lower acids through the action of the excess of mine on the amines produced :—

$$C_nH_{2n+1}.CH_2.NBr_2 = C_nH_{2n+1}.CN + 2HBr \text{ ; and,}$$
$$C_nH_{2n+1}.CN + 2H_2O = C_nH_{2n+1}.COOH + NH_3 .$$

A modified reaction occurs when amido-compounds are treated once with a solution of sodium hypobromite containing an excess of caustic soda. Under these circumstances the nitrogen is in some cases wholly or partly evolved as gas, a fact utilised for the determination of urea, &c.

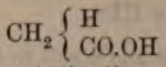
The amides, though derivatives of ammonia, have very feebly-marked basic characters, the basylous radical being apparently neutralised by the chlorous group; indeed, one molecule may be said to neutralise another. The primary amides are, however, capable of forming hydrochlorides [*e.g.*, acetamide hydrochloride, $(C_2H_3O.NH_2)_2HCl$] and certain other salts, but such compounds are very unstable, being decomposed in most cases by water alone (compare acetanilide). On the other hand, the hydrogen of the NH_2-group can be replaced by metals, especially mercury, the amide existing as an acid in such compounds.

The amides are remarkable for their high melting and boiling-points as compared with the corresponding amines. Thus acetamide, $C_2H_3O.NH_2$, does not *melt* below 222° C., whereas ethylamine, $C_2H_5.NH_2$, *boils* at 19°.

By the exchange of the oxygen in amides for the imido-residue, NH, *amidines* or amido-amines are formed, of which class the typical substance is acetamidine:—$CH_3.C(NH).NH_2$.

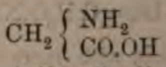
The amidines have well-characterised basic properties, but are very easily saponified by boiling either with alkalies or with acids.

Amido-acids are compounds in which NH_2 replaces an atom the hydrogen of the *methyl*-group, whereas in the amides the O of the *carboxyl*-group is replaced, as shown by the followi formulæ:—



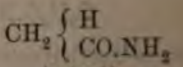
$$CH_2\begin{cases}H\\CO.OH\end{cases}$$

Acetic acid.



$$CH_2\begin{cases}NH_2\\CO.OH\end{cases}$$

Amido-acetic acid.



$$CH_2\begin{cases}H\\CO.NH_2\end{cases}$$

Acetamide.

The amido-acids result from the replacement of one of th hydrogen-atoms in direct union with carbon by the amido-grou NH_2, which can be effected, among other methods, by treatin the chloro- or bromo-acid with ammonia:—

$$\underset{\text{Bromacetic acid.}}{CH_2Br.COOH} + NH_3 = HBr + \underset{\text{Amidoacetic acid.}}{CH_2(NH_2).COOH}.$$

On the other hand, when the *hydroxyl* of the acid is replace by an amido-group an acid amide is formed:—

$$\underset{\text{Acetyl chloride.}}{CH_3.CO.Cl} + NH_3 = HCl + \underset{\text{Acetamide.}}{CH_3.CO(NH_2)}.$$

Amic acids are derivatives of polybasic acids intermediat between the amido-acids and amides. They contain both a

ACID.	(H replaced by NH_2).	(OH replaced by NH_2).	(CO.OH replaced by $CO.NH_2$).
$\left\{\begin{matrix}CH_3\\CO.OH\end{matrix}\right.$ Acetic acid.	$\left\{\begin{matrix}CH_2.NH_2\\CO.OH\end{matrix}\right.$ Amidoacetic acid. *Glycocine.*	... ...	$\left\{\begin{matrix}CH_3\\CO.NH_2\end{matrix}\right.$ Acetamide.
$\left\{\begin{matrix}CH\left\{\begin{matrix}H\\OH\end{matrix}\right.\\CO.OH\end{matrix}\right.$ Hydroxyacetic acid. *Glycollic acid.*	$\left\{\begin{matrix}CH\left\{\begin{matrix}NH_2\\OH\end{matrix}\right.\\CO.OH\end{matrix}\right.$ Amidoglycollic acid.	$\left\{\begin{matrix}CH\left\{\begin{matrix}H\\NH_2\end{matrix}\right.\\CO.OH\end{matrix}\right.$ Glycolamic acid. *Glycocine.*	$\left\{\begin{matrix}CH\left\{\begin{matrix}H\\OH\end{matrix}\right.\\CO.NH_2\end{matrix}\right.$ Glycolylamide.
$\left\{\begin{matrix}C_2H_3\left\{\begin{matrix}H\\OH\end{matrix}\right.\\CO.OH\end{matrix}\right.$ Hydroxypropionic acid. *Lactic acid.*	$\left\{\begin{matrix}C_2H_3\left\{\begin{matrix}NH_2\\OH\end{matrix}\right.\\CO.OH\end{matrix}\right.$ Amidolactic acid. *Serine.*	$\left\{\begin{matrix}C_2H_3\left\{\begin{matrix}H\\NH_2\end{matrix}\right.\\CO.OH\end{matrix}\right.$ Lactamic acid. *Alanine.*	$\left\{\begin{matrix}C_2H_3\left\{\begin{matrix}H\\OH\end{matrix}\right.\\CO.NH_2\end{matrix}\right.$ Lactamide.
$\left\{\begin{matrix}OH\\CO.OH\end{matrix}\right.$ Carbonic acid.	... ...	$\left\{\begin{matrix}NH_2\\CO.OH\end{matrix}\right.$ Carbamic acid.	$\left\{\begin{matrix}NH_2\\CO.NH_2\end{matrix}\right.$ Carbamide. *Urea.*
$\left\{\begin{matrix}CO.OH\\CO.OH\end{matrix}\right.$ Oxalic acid.	... ...	$\left\{\begin{matrix}CO.NH_2\\CO.OH\end{matrix}\right.$ Oxamic acid.	$\left\{\begin{matrix}CO.NH_2\\CO.NH_2\end{matrix}\right.$ Oxamide.
$C_2H_4\left\{\begin{matrix}CO.OH\\CO.OH\end{matrix}\right.$ Succinic acid.	$C_2H_3(NH_2)\left\{\begin{matrix}CO.OH\\CO.OH\end{matrix}\right.$ Amidosuccinic acid. *Aspartic acid.*	$C_2H_3(NH_2)\left\{\begin{matrix}CO.NH_2\\CO.OH\end{matrix}\right.$ Amidosuccinamic acid. *Asparagine.*	$C_2H_4\left\{\begin{matrix}CO.NH_2\\CO.NH_2\end{matrix}\right.$ Succinamide.
$C_2H_3(OH)\left\{\begin{matrix}CO.OH\\CO.OH\end{matrix}\right.$ Malic acid.	... ...	... ...	$C_2H_3(OH)\left\{\begin{matrix}CO.NH_2\\CO.NH_2\end{matrix}\right.$ Malamide.
$C_2H_2(OH)\left\{\begin{matrix}CO.OH\\CO.OH\end{matrix}\right.$ Tartaric acid.	... ...	$C_2H_2(OH)_2\left\{\begin{matrix}CO.NH_2\\CO.OH\end{matrix}\right.$ Tartramic acid.	$C_2H_2(OH)_2\left\{\begin{matrix}CO.NH_2\\CO.NH_2\end{matrix}\right.$ Tartramide.

amidogen-group, NH_2, and a carboxyl-group, COOH, whereas the latter is absent from the corresponding amides.

By treating amido-acids and amic acids with nitrous acid, the corresponding hydroxy-acids are formed and nitrogen evolved. Thus, aspartic acid (succinamic acid) yields hydroxy-succinic acid (malic acid) by reaction with nitrous acid. Amidoacetic acid (glycocine) yields hydroxy-acetic acid (glycollic acid).

The table on the preceding page shows the formulæ and relationships of some of the more important bodies of the amido-group. Among other interesting synthetical bodies of analogous constitution are :—

Acetyl-glycocine, .	. *Aceturic acid*, .	. $\left\{\begin{matrix} CH_2.NH(C_2H_3O) \\ CO.OH \end{matrix}\right.$
Ethyl carbamate, .	. *Urethane*, .	. $\left\{\begin{matrix} NH_2 \\ CO.O(C_2H_5) \end{matrix}\right.$
Phenyl-urethane, .	. *Euphorin*, .	. $\left\{\begin{matrix} NH(C_6H_5) \\ CO.O(C_2H_5) \end{matrix}\right.$

A number of interesting natural compounds also belong to the same class. Among these may be mentioned leucine, tyrosine, asparagine, glutamine, cystin, betaïne, choline, neurine, an muscarine. These are formulated and described on page 21 *et seq.* Urea is considered in a separate section (page 248).

Glycocine. Glycocoll. Glycine. Amido-acetic acid.

$$C_2H_5NO_2;\ i.e.,\ N'\left\{\begin{matrix} H_3 \\ CH_2.CO \\ O\ \text{——} \end{matrix}\right.\left.\begin{matrix} \\ \\ \\ \end{matrix}\right\};\ \text{or}\ N'''\left\{\begin{matrix} H \\ H \\ CH_2.COOH \end{matrix}\right.$$

Glycocine does not appear to occur frequently ready-form in nature, though it is said to occur in the muscle of *Pec* *irradians.* Glycocine is a very frequent product of the action acids or alkalies on animal matters. Thus it was first obtaine by Braconnot in 1820 by boiling glue with sulphuric aci whence its name of glycocoll or sugar of gelatin. It lik wise results from the action of caustic alkalies on meat or gelati and is also formed when hippuric acid is boiled with hydrochlor acid, or when glycocholic or hyoglycocholic acid is boiled wi baryta-water.

$$\underset{\text{Hippuric acid.}}{(C_7H_5O).NH.CH_2.COOH} + OH_2 = \underset{\text{Benzoic acid.}}{C_7H_5O.OH} + \underset{\text{Glycocine.}}{NH_2.CH_2.COOH}$$

$$\underset{\text{Glycocholic acid.}}{(C_{24}H_{39}O_4).NH.CH_2.COOH} + OH_2 = \underset{\text{Cholic acid.}}{C_{24}H_{40}O_5} + \underset{\text{Glycocine.}}{NH_2.CH_2.COOH}$$

Glycocine is best prepared by boiling hippuric acid for half a hour with four parts of fuming hydrochloric acid. The produc is diluted with water and allowed to cool, when the greater part of the benzoic acid crystallises out. The remainder is removed by agitation with ether or petroleum-spirit, and the solution of glycocine hydrochloride evaporated till the salt crystallises on cooling. It is washed with absolute alcohol, and on treatment with an equivalent amount of litharge or oxide of silver yields free glycocine, which is recrystallised from water or dilute spirit.

Glycocine may be prepared by boiling glycocholic acid for twelve hours with strong hydrochloric acid, filtering from the resinous mixture of cholic acid and dyslysin, and evaporating the filtrate. The glycocine hydrochloride is dissolved in water and treated with lead hydroxide, the liquid filtered, and the soluble lead compound of glycocine decomposed by passing sulphuretted hydrogen. On concentrating the filtered liquid, glycocine is deposited in crystals.

Glycocine may also be prepared by boiling a concentrated solution of chloracetic or bromacetic acid with a large excess of strong ammonia.

Glycocine has the constitution of an amidoacetic acid, and is the type of a somewhat numerous class of substances which either actually occur in the animal tissues or products of their change, or are closely related to such compounds of natural occurrence.

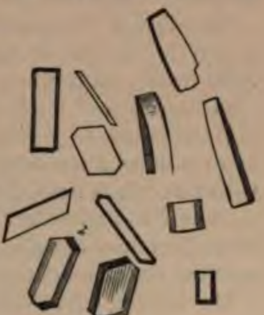


Fig. 1.—Glycocine.

Glycocine forms very hard, flattened prisms or aggregated plates, belonging to the monoclinic system (fig. 1).[1] The crystals grate between the teeth and have a sweet taste, but are not poisonous. When heated to 170° C., glycocine melts, and at a higher temperature decomposes with separation of carbon.

Glycocine dissolves in about 400 parts of cold and a smaller quantity of boiling water. It crystallises readily by spontaneous evaporation of its aqueous solution. It is moderately soluble in rectified spirit, but insoluble in absolute alcohol, even when boiling, as also in ether. Glycocine is optically inactive. It is not susceptible of the alcoholic fermentation.

When glycocine is boiled with *concentrated* caustic alkali it evolves ammonia,[1] and on treating the residue with hydrochloric acid hydrocyanic acid is disengaged, while oxalic acid is found in the liquid.

---

[1] Mere traces of impurities are stated to influence the crystalline form and other physical characters of glycocine in a remarkable manner.

Glycocine is charred by strong sulphuric acid. Distilled with dilute sulphuric acid and manganese or lead dioxide, it yields hydrocyanic and carbonic acids:—

$$C_2H_5NO_2+O_2=2H_2O+CO_2+HCN$$

Nitrous acid converts glycocine into glycollic acid, thus:—

$$CH_2(NH_2).COOH+HNO_2=CH_2(OH).COOH+H_2O+N_2$$

On agitating the liquid with ether, the glycollic acid is dissolved.[2]

Glycocine evolves no nitrogen when treated with an alkaline solution of sodium hypobromite (A. H. Allen).

On addition of mercurous nitrate, cold solutions of glycocine yield a grey precipitate of metallic mercury, but the reaction occurs more readily on heating.

On addition of ferric chloride to a solution of glycocine, a strong red coloration is produced. This is destroyed by acids, but reappears on cautious neutralisation. Hence, amidoacetic acid reacts with ferric salts much like acetic acid itself.

When treated with a drop of phenol and then with a solution of sodium hypochlorite, glycocine gives a blue coloration, in this reaction resembling ammonia, aniline, and methylamine.

On heating glycocine in a sealed tube with benzoic acid, hippuric acid is produced. The same product is formed by treating glycocine with hydrochloric acid and benzoyl chloride. This reaction has been proposed by C. S. Fischer (*Zeit. physiol. Chem.*, xix. 164) for the determination of glycocine, and the value of the method has been confirmed by M. Gonnermann (*Pflüger's Archiv*, 1894, lix. 42). When glycocine is taken internally, it appears in the urine as hippuric acid, which has the constitution of a benzoyl-glycocine, $(C_6H_5.CO).NH.CH_2.COOH$.

Glycocine is neutral to litmus, and has at once the characters of an acid and a base.[1] It combines with metallic oxides, and forms crystallisable salts with acids. On boiling an aqueous solution of glycocine with cupric hydroxide or acetate, cupric amidoacetate, $Cu(C_2H_4NO_2)_2+H_2O$, separates in fine blue needles on cooling or on adding alcohol. The compound dissolves in caustic alkalies with deep blue colour. If glycocine and caustic potash be added to a solution of cupric sulphate, the liquid becomes dark blue, and the above salt is precipitated on adding alcohol.

---

[1] According to Horsford a fiery-red coloration is produced by heating glycocine with strong alkalies, but the reaction appears to have been due to an impurity.

[2] On separating and evaporating the solution, the glycollic acid is obtained in fine laminæ, which are unchanged in the air, melt at 80°, and are readily soluble in alcohol.

GLYCOLLIC ACID, $CH_2(OH).COOH$, forms crystallisable salts, most of which are readily soluble. The neutral *lead* salt is soluble in cold water, but on boiling its solution, or on precipitating a glycollate with lead acetate, a basic salt, $(C_2H_3O_3Pb)_2O$, separates in stellar needles, requiring 10,000 parts of water for solution. *Cupric glycollate*, $Cu(C_2H_3O_3)_2$, forms blue crystals, which require 134 parts of cold water for solution.

The barium, strontium, calcium, and magnesium salts of amidoacetic acid have been obtained in a crystallised state (*Annalen*, cclxvi. 292), and the mercury, lead, cadmium, and palladium salts are likewise crystalline. The silver salt crystallises in tablets, has a strong alkaline reaction, turns grey in the light, and decomposes at 100° C.

In addition to mere homologues, several important and interesting derivatives of glycocine occur naturally in the animal kingdom. Among these bodies are hippuric and salicyluric acids, occurring in urine; glycocholic acid and hyoglycocholic acid, constituents of bile; glycocyamine, creatine, &c.

Glycocine hydrochloride, $C_2H_5NO_2,HCl$, forms deliquescent crystals, having an acid reaction and astringent taste. It is readily soluble in water, but only slightly in alcohol. B_2,HCl forms trimetric crystals. B_2,H_2SO_4 forms large non-deliquescent prisms, soluble in water, but insoluble in alcohol or ether. B,HNO_3 and $B_2,H_2C_2O_4$ are also crystallisable.

In consequence of the double function exerted by glycocine, it not only combines both with bases and acids, but forms a peculiar class of compounds with neutral salts. Of these the type is the potassium nitrate compound :—$NO_3.NH_3.CH_2.COOK$.

Analogues of Glycocine.

Glycocine may be looked on as the starting point or type of two distinct series of bases, according as it is regarded as an amido-acid or an internal anhydride[1] :—

$$C\left\{\begin{array}{l} NH_2 \\ H \\ H \\ CO.OH \end{array}\right. \qquad \text{Glycocine.} \qquad N\left\{\begin{array}{l} H \\ H \\ H \\ \left.\begin{array}{l} CH_2.CO \\ O \text{———} \end{array}\right\} \end{array}\right.$$

Amido-acid. Internal Anhydride.

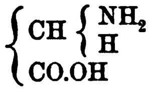
---

[1] It has been suggested that free glycocine may be regarded as glycocine amidoacetate:—$H_3N\left\{\begin{array}{c} O.CO.CH_2 \\ CH_2.CO.O \end{array}\right\}NH_3$. A determination of the molecular weight of glycocine in aqueous solution, by Raoult's method, does not confirm this doubled formula.

Alanine, butalanine, and leucine are bases of the first type; sarcosine and betaine belong to the second class, and together with neurine and choline are considered in a separate section under the head of Betaïnes (page 232).

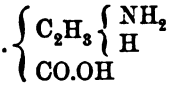
The chief members of the first series of analogues of glycocine may be thus formulated:—

Glycocine. Amidoacetic acid, . .



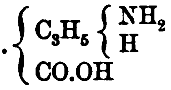
$$\left\{\begin{array}{l} CH\left\{\begin{array}{l} NH_2 \\ H \end{array}\right. \\ CO.OH \end{array}\right.$$

Alanine. Amidopropionic acid, . .



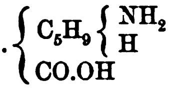
$$\left\{\begin{array}{l} C_2H_3\left\{\begin{array}{l} NH_2 \\ H \end{array}\right. \\ CO.OH \end{array}\right.$$

Butalanine. }
Piperic Acid. } Amidobutyric acids, .



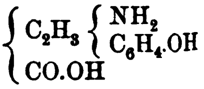
$$\left\{\begin{array}{l} C_3H_5\left\{\begin{array}{l} NH_2 \\ H \end{array}\right. \\ CO.OH \end{array}\right.$$

Leucine. Amidocaproic acid, . .



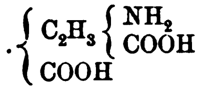
$$\left\{\begin{array}{l} C_5H_9\left\{\begin{array}{l} NH_2 \\ H \end{array}\right. \\ CO.OH \end{array}\right.$$

Tyrosine. Parahydroxyphenyl-amidopropionic acid, . .



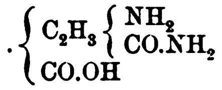
$$\left\{\begin{array}{l} C_2H_3\left\{\begin{array}{l} NH_2 \\ C_6H_4.OH \end{array}\right. \\ CO.OH \end{array}\right.$$

Aspartic Acid. Amidosuccinic acid, .



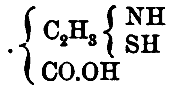
$$\left\{\begin{array}{l} C_2H_3\left\{\begin{array}{l} NH_2 \\ COOH \end{array}\right. \\ COOH \end{array}\right.$$

Asparagin. Amidosuccinamic acid, .



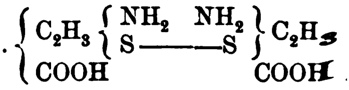
$$\left\{\begin{array}{l} C_2H_3\left\{\begin{array}{l} NH_2 \\ CO.NH_2 \end{array}\right. \\ CO.OH \end{array}\right.$$

Cysteïn. Amidothiolactic acid, . .



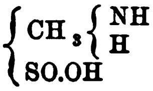
$$\left\{\begin{array}{l} C_2H_3\left\{\begin{array}{l} NH \\ SH \end{array}\right. \\ CO.OH \end{array}\right.$$

Cystin, ...



$$\left\{\begin{array}{l} C_2H_3\left\{\begin{array}{l} NH_2 \quad\quad NH_2 \\ S \text{———} S \end{array}\right\}C_2H_3 \\ COOH \quad\quad\quad\quad\quad\quad COOH \end{array}\right.$$

Taurin. Amidoethane-sulphonic acid, .



$$\left\{\begin{array}{l} CH_3\left\{\begin{array}{l} NH \\ H \end{array}\right. \\ SO.OH \end{array}\right.$$

It will be observed that alanine, tyrosine, aspartic acid, asparagin, stin, cysteïn, and taurin are closely related, all containing the oup—$C_2H_3(NH_2)$. Glycocine is a lower homologue of alanine, nd butalanine and leucine higher homologues of the same series.

Leucine. Amidocaproic Acid. α-Amido-*n*-hexoic acid.

$$C_6H_{13}NO_2;\ i.e.,\ C_4H_9.CH:\left\{\begin{array}{l}NH_2\\CO.OH.\end{array}\right.$$

Leucine was originally discovered by Proust (1819) in putrefied cheese. It was found by Liebig in diseased, but not in healthy, alf's liver. It has been found in the brain, pancreas, thyroid and hymoid glands, &c.; and in the liver and urine in cases of small-ox, typhus fever, leucæmia, affections of the spinal cord, and oisoning by phosphorus; also in many invertebrate animals. eucine is a characteristic product of the putrefaction of gelatin and roteids, and is produced, together ith glycocine, tyrosine, as-artic and glutamic acids, by the ction of boiling dilute acids or used caustic potash on these ubstances.

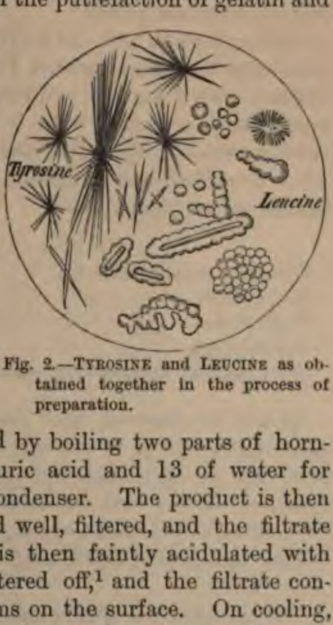


Fig. 2.—Tyrosine and Leucine as obtained together in the process of preparation.

In the vegetable kingdom, ucine has been found in young umpkins, beetroot and beetroot olasses, the juice of vetches erminated in the dark; in *Agari-us muscarius*, &c., &c.

Leucine has been prepared nthetically by the reaction of -bromohexoic acid with ammonia.

Leucine is conveniently prepared by boiling two parts of horn-havings with five parts of sulphuric acid and 13 of water for wenty-four hours, under a reflux condenser. The product is then reated with excess of lime, boiled well, filtered, and the filtrate vaporated to about one-half. It is then faintly acidulated with xalic acid, the calcium oxalate filtered off,[1] and the filtrate con-entrated till a crystalline film forms on the surface. On cooling, rystalline laminæ are deposited, consisting of a mixture of leucine nd tyrosine (fig. 2), and a further crop can be obtained by concentrating the mother-liquor. The crystals are redissolved in such a quantity of boiling water that only tyrosine is deposited on cooling. The mother-liquor is treated with precipitated hydroxide of lead, which removes colouring matter and a little tyrosine, and the filtrate freed from lead by sulphuretted hydrogen and evaporated till a film forms on the surface. The crystals of leucine which deposit on cooling may be further purified from tyrosine by treatment with boiling alcohol of 70 per cent. (sp. gr., 0·872), which leaves the tyrosine undissolved. To get rid of traces of a sulphuretted impurity, the leucine may be dissolved in dilute caustic alkali, a solution of lead oxide in caustic alkali added, and the liquid boiled for half an hour. The liquid filtered from the lead sulphide is exactly neutralised by sulphuric acid, evaporated to complete dryness, and the residue exhausted with boiling alcohol of 0·830 specific gravity, which on cooling deposits the leucine in a state of absolute purity.[1]

---

[1] At this stage Waage gradually adds recently precipitated cupric hy-roxide (avoiding excess), and boils. On cooling, a copper compound of ucine separates out in light blue scales. This is filtered off, decomposed by ulphuretted hydrogen, and the liberated leucine purified by crystallisation rom dilute alcohol.

Erlenmeyer and Schöffer have recorded the quantities o leucine and tyrosine yielded by boiling one part of various anim matters with 5 parts of a diluted acid made by mixing 2 parts o strong sulphuric acid with 3 parts of water. They found th results equally good after three hours' boiling with the acid a when treatment was continued for 36 to 40 hours. The followin results were obtained :—

	Leucine.		Tyrosine	
Cervical ligament of the ox, . .	38–45	per cent.	¼	per cent.
Blood fibrin, ...	14	,,	2	,,
Flesh fibrin, ...	18	,,	1	,,
White of egg, ...	10	,,	1	,,
Horn (with 10 parts of dilute acid), .	10	,,	3·6	,,

It will be observed that the yield of leucine from the cervical ligament of the ox is not only much greater than from horn, but that only an insignificant proportion of tyrosine is produced. The ligament should first be thoroughly freed from fat by treatment with ether or petroleum-spirit, and from adhering fibrous tissue by boiling with water containing acetic acid. The leucine obtained by concentrating the filtrate from the calcium oxalate precipitate from this source is fairly pure after being recrystallised once or twice from dilute alcohol.

---

[1] For the extraction of the traces of leucine and tyrosine existent in urine, the liquid should be treated with basic lead acetate, filtered, the excess of lead removed by sulphuretted hydrogen, and the filtrate evaporated to dryness. The leucine is dissolved by treating the residue with boiling alcohol, while the tyrosine, which remains insoluble, is crystallised from slightly ammoniacal water. Very minute traces of leucine and tyrosine exist in normal urine, but in phosphorus poisoning and acute yellow atrophy of the liver the proportion is greatly increased.

Leucine separates from alcohol in thin nacreous scales resembling cholesterin. The crystals have a specific gravity of 1·293, but are wetted with difficulty by water, and often float on the surface. When not perfectly pure, leucine often separates in concentric nodules closely resembling fatty cells (fig. 4), but which under the microscope appear as concentrically grouped, highly refracting needles.

When cautiously heated (in a tube open at both ends), leucine sublimes unchanged in light white flocks, which under the microscope are seen to consist of delicate scales grouped together in the form of rosettes. When heated to 170°, leucine melts to a brown

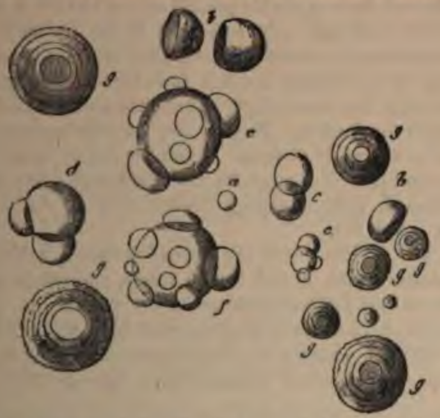


Fig. 3.—Spheroidal crystalline masses of leucine. *a*, a very minute simple spherule; *b*, hemispheroidal masses; *c c*, aggregates of small globules; *d*, a large globule supporting two halves; *e f*, a large spheroid of leucine richly studded with minute segments; *g g g g*, laminated globules of leucine, some with smooth, some with rough surface, and of very various sizes.

viscous liquid, and at a slightly higher temperature decomposes into amylamine and carbon dioxide:—$C_6H_{13}NO_2 = C_5H_{13}N + CO_2$.

Leucine is sparingly soluble in cold water (1 : 49 at 12°, Hüfner; 1 : 45 at 18°, Schulze; 1 : 27, Zollikofer), but more readily in boiling water. It requires 660 parts of cold rectified spirit and 1040 parts of cold alcohol of 96 per cent. for solution, but dissolves in 800 parts of boiling 98 per cent. alcohol, and more readily in weaker spirit. Its solubility in water and in alcohol is increased by acetic acid or an acetate of alkali-metal. Leucine is insoluble in ether. It dissolves readily in acids and alkalies.

Leucine is optically active, the specific rotatory power for the Fraunhöfer line D being stated by Peese to be +14·1° for a 15 per cent. solution in hydrochloric acid, and +5·6° for a 25 per cent. solution in ammonia.[1]

A variety of leucine has been obtained by Schulze and Likiernik from the vegetable albuminoid conglutin. When heated with baryta to 160° it loses its optical activity, and forms an inactive modification which has been proved to be identical with the α-amido-isobutylacetic acid prepared synthetically from iso-valeraldehyde. Both compounds have the same solubility in water, yield active leucine under the action of *Penicillium glaucum*, and are converted into the same hydroxycaproic acid on treatment with nitrous acid.[2]

[1] According to Schulze and Bosshard (*Zeit. physiol. Chem.*, x. 134), the leucine obtained from vegetable proteid conglutin is optically active when hydrochloric acid has been employed as the decomposing agent, but inactive if baryta-water at a temperature of 150°–160° has been used. When leucine obtained by the former method is heated with baryta-water under pressure at 150°–160° for three days, it becomes optically inactive, and its solubility in water diminishes. Heating leucine with water alone under the same conditions produced no change. When the mould *Penicillium glaucum* was grown in a solution of leucine for five or six weeks, the optical activity was exactly reversed, $[\alpha]_D$ changing from +17·3° to −17·3° (See also Schulze, *Ber.*, xxvi. 56).

[2] Schulze and Likiernik (*Zeit. physiol. Chem.*, xvii. 513) find that the leucine obtained from fibrin, elastic tissue, and other sources has the same properties as that derived from conglutin.

On the other hand, B. Gmelin (*Zeit. physiol. Chem.*, xviii. 21) finds the leucine from different sources to vary in solubility and optical activity, though identical in elementary composition. Gmelin gives the following data :—

Source of Base.	Solubility in Water.		Sp. Rotation in HCl Solution. $[\alpha]_D=$	Melting-point of Leucic Acid.
	At 19° C.	At about 100°.		
Yeast, . .	1 in 28·8	1 in 15·9	+ 17·2°	72·5°
Casein, . .	1 in 29·0	1 in 14·3	+ 17·2°	72·5°
Hæmoglobin,	1 in 45·8	1 in 18·7	+ 14·3°	67·0°

According to R. Cohn (*Ber.*, 1894, xxvii. 2727), leucine obtained by fermenting blood-fibrin with calf's pancreas does not melt and partially sublimes at 170°, but melts and decomposes at 275°–276°. This is about the melting-point of inactive leucine, from which the new modification differs by its greater solubility in water, in being feebly lævo-rotatory, and in being completely destroyed by *Penicillium glaucum*.

When fused with caustic alkali, leucine yields normal valeric cid, $C_5H_{10}O_2$, ammonia, hydrogen, and carbon dioxide. When eated with fuming hydriodic acid to 140° it yields caproic cid and ammonia, $C_6H_{13}NO_2 + H_2 = C_6H_{12}O_2 + NH_3$. Nitrous cid decomposes leucine into nitrogen and hydroxycaproic r leucic acid, $C_5H_{10}(OH).CO.OH$.

When leucine is heated with nitric acid on platinum foil, a olourless residue is left, which becomes yellow on addition of a drop of caustic soda; and on careful evaporation this forms an oily drop which does not wet the platinum. This reaction was observed by Scherer (*Jahresber.*, 1857, 541), and is said to be characteristic.

When heated for some time with excess of nitric acid, leucine is entirely converted into gaseous products; but as long as the decomposition is incomplete the remaining portion consists of nitrate of leucine.

Heated with strong sulphuric acid, leucine is decomposed, the whole of the nitrogen being converted into ammonia. By potassium permanganate it is converted into valeric acid, oxalic acid, and mmonia. The two preceding reactions have a practical interest n connection with Kjeldahl's process of determining nitrogen n albuminoid matters.

An aqueous solution of leucine emits an offensive odour, and oon becomes acid. The change does not occur in a vacuum.

An aqueous solution of leucine is coloured deep red by ferric hloride.

Leucine is neutral in reaction, but in its capacity of an amido-cid forms crystallisable compounds both with acids and bases, nd also unites with neutral salts in a manner similar to glycocine page 209).

B,HCl forms crystalline scales, very soluble in water. B_2,H_2PtCl_6 orms yellow crystalline grains, soluble in water, but insoluble in lcohol. B,HNO_3 forms colourless needles, very readily soluble in ater.

Copper amido-caproate, $Cu(C_6H_{12}NO_2)_2$, is obtained by adding ecently precipitated cupric hydroxide to a strong aqueous solu-on of leucine, and boiling the liquid. A bluish solution results, hich on cooling deposits light blue scales, which require 3045 arts of cold or 1460 parts of boiling water for solution. This eaction may be employed for the isolation of leucine, but the olubility of the copper compound is materially increased by the resence of certain organic matters. With excess of copper oxide, ucine forms an insoluble compound. $Pb(C_6H_{12}NO_2)_2 + H_2O$ is eposited in mirror-like plates or nacreous scales, on cautiously dding ammonia to a boiling solution of leucine to which lead acetate has been added. Mercurous nitrate is said to precipitate leucine in white flocks, the supernatant liquor acquiring a red colour if tyrosine be present.

Tyrosine. Para-hydroxyphenyl-α-amidopropionic Acid.

$$C_9H_{11}NO_3\text{; } i.e., \left\{\begin{array}{l} C_2H_3(NH_2).COOH \\ C_6H_4(OH) \end{array}\right.$$

Tyrosine occurs ready-formed, and almost always accompanied by leucine, in both the animal and vegetable organisms.[1] It is likewise produced, together with leucine, by the putrefactive decomposition of proteids, or by their treatment with alkalies or acids, and has also been obtained synthetically.

The proportions of tyrosine and leucine yielded on boiling certain animal matters with diluted sulphuric acid are given on page 212.

A method of preparing tyrosine is described on page 211. Another plan is to gradually add dry casein, fibrin, or albumin (free from fat) to an equal weight of fused or highly concentrated solution of caustic potash contained in a capacious iron vessel. The heating, which is accompanied by an evolution of ammonia and most disagreeable odour, is continued until evolution of hydrogen commences, and the fused mass changes in colour from brown to yellow. The product is then poured out, dissolved in hot water, and the solution slightly acidulated with acetic acid. On cooling an abundant crop of crystals of tyrosine separates in concentric groups of needles,[2] which may be purified by re-solution in hot water containing potassium carbonate and precipitation with acetic acid.

For the further purification of tyrosine, it should be dissolved in a known quantity of hydrochloric acid, the solution treated with animal charcoal, and filtered. The hot filtrate is treated with sodium or potassium acetate in amount equivalent to the hydrochloric acid used, when the greater part of the tyrosine separates on cooling. A product much freer from inorganic matter is obtainable in this way than when crystallisation is effected in a neutral solution. The tyrosine should be recrystallised from hot water containing acetic acid. For the removal of an obstinately adhering sulphuretted impurity, Städeler adds to the warm aqueous solution a small quantity of basic lead acetate, treats the filtered liquid with sulphuretted hydrogen, and recovers the tyrosine by concentrating the filtrate to the crystallising point.

---

[1] Tyrosine is said to occur with leucine in spiders, caterpillars, and moths; but not in butterflies, which contain leucine only. It occurs in the cochineal insect, and in the pancreas and other organs of mammals.

[2] Leucine may be obtained by concentrating the mother-liquor.

Tyrosine is deposited from its hot aqueous solution in stellate groups of long, slender, silky needles, which on drying readily become felted together to a snow-white mass. From ammoniacal solutions it is deposited in larger needles, also having a silky lustre (fig. 4).

Tyrosine is tasteless, odourless, and infusible. When heated, it evolves an odour of burnt bones. Heated cautiously to 270°, it gives off carbon dioxide, and yields a white sublimate of hydroxyethyl-aniline, $C_2H_4(OH).NH.C_6H_5$.

Tyrosine is soluble in 2400 parts of cold or 150 parts of boiling water. In hot ammonia and in acetic acid it dissolves unchanged, and is deposited on cooling. It requires 13,500 parts of cold rectified spirit for solution, is not much more soluble on boiling, and is quite insoluble in absolute alcohol and in ether. Its solubility in alcohol is greatly increased by the presence of amorphous extractive matters.

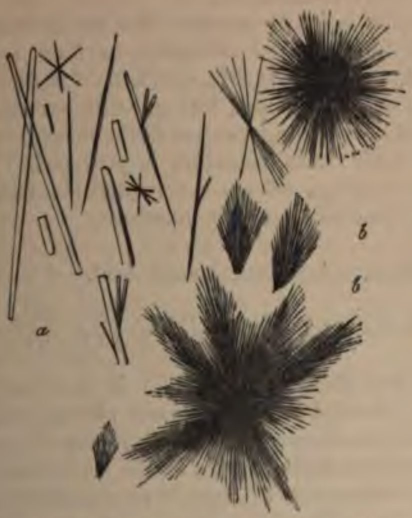


Fig. 4.—Tyrosine. *a*, single crystals; *b b*, smaller and larger groups.

Tyrosine is lævo-rotatory. The value of $[\alpha]_D$ in solution in fuming hydrochloric acid is $-7{\cdot}98°$; in solution in 12 per cent. caustic potash, $-9{\cdot}0°$.

When fused with caustic potash, tyrosine yields ammonia, acetic acid, and para-hydroxybenzoic acid, $C_6H_4(OH).COOH$.

When treated with strong nitric acid, tyrosine is converted into nitrotyrosine nitrate, from the solution of which ammonia precipitates free nitrotyrosine, $C_9H_{10}(NO_2)NO_3$, which crystallises from hot water in light yellow, very sparingly soluble needles, having a slightly bitter but not acid taste, and dissolving in caustic alkalies with deep red colour. Dinitrotyrosine, $C_9H_9(NO_2)_2NO_3$, is obtained by evaporating tyrosine with excess of nitric acid. It is a well-defined dibasic acid, forming golden-yellow plates, sparingly soluble in water, but readily in alcohol, having an acid but not bitter taste. The salts deflagrate on heating.

If Millon's reagent be added to a boiling aqueous solution of tyrosine, the liquid acquires a pink or rose-red colour, and red flakes are gradually precipitated.

When tyrosine is gently warmed with strong sulphuric acid, it dissolves with transient red coloration to form tyrosine-sulphonic acid, $C_9H_{10}(SO_3H)NO_3+2H_2O$. On diluting the solution after a time with water, boiling with barium carbonate or chalk, and gradually adding neutral ferric chloride to the neutral filtrate, a fine dark violet coloration is produced. This reaction, which is due to Piria, affords a very delicate test for tyrosine. Unfortunately, leucine somewhat interferes.

Asparagine. Amidosuccinamic Acid.

$$C_4H_8N_2O_3\,;\ \textit{i.e.},\ C_2H_3(NH_2)\begin{cases}CO.NH_2\\CO.OH\end{cases}$$

Asparagine was discovered in 1805 in the juice of asparagus. It exists ready-formed in many other plants, including marsh-mallow, comfrey, chestnuts, potatoes, the leaves of the deadly night-shade, liquorice-root, dahlia tubers, and is present in comparatively large quantity in the roots of *Robinia pseudacacia*. Asparagine is also found in the milky juice of the lettuce, and in the young shoots of vetches, beans, peas, and other leguminous plants, though the *seeds* of these contain no trace of asparagine. The quantity of asparagine diminishes with the progress of the growth of the plant, and disappears entirely when the seeds are formed. Boussingault finds asparagine to be constantly present in plants grown in the dark.

Asparagine may be prepared by dialysing the juice of asparagus, marsh-mallow, or *Scorzonera Hispanica*, concentrating the dialysate to a syrup, and allowing it to stand for some days, when the asparagine will separate in crystals. From liquorice, asparagine may be prepared by exhausting the root with water, boiling to coagulate albumin, treating the filtrate with acetic acid to precipitate glycyrrhizic acid, and adding lead acetate to the filtered liquid, to throw down phosphates, malates, colouring matter, &c. The filtrate, when evaporated to a small bulk, deposits crystals of asparagine after standing for some days. Asparagine may also be isolated by treating the filtrate from the lead precipitate with mercuric nitrate, and decomposing the resultant compound with sulphuretted hydrogen. This plan is especially useful in the presence of soluble carbohydrates, which prevent the crystallisation of the asparagine.

Asparagine forms hard, transparent, rhombic prisms, which have a specific gravity of 1·519. The crystals belong to the trimetric system, and exhibit left-handed hemihedry. They contain one molecule of water which is lost at 100° C. The crystals grate between the teeth, and have a slightly cooling, sickly taste.

Asparagine is moderately soluble in cold water (1 : 82 at 10° C. ; 1 : 47 at 20°), but much more readily on boiling (1 : 1·9).[1] It dissolves freely in acid and alkaline liquids. It is insoluble in cold absolute alcohol, and almost insoluble on boiling, and is not dissolved by ether, or by fixed or volatile oils.

Asparagine is optically active, but the extent and direction of he rotation depend on the solvent. Thus a solution of asparagine water has a specific rotation of about −6° ; but by addition of kalies the activity is increased, in ammoniacal solution the rotan being −11°. In hydrochloric acid solution, on the other nd, asparagine exhibits a dextro-rotation of about +36°. ddition of a small quantity of acetic acid to the aqueous solution asparagine decreases the lævo-rotation, and on further addition acid the liquid becomes dextro-rotatory.[2]

The aqueous solution of asparagine is stated to have a faintly id reaction to litmus. When quite pure it can be kept without ange, but in presence of albuminous matter or other impurity, e solution soon ferments, the asparagine being completely conrted into ammonium succinate:—

$$C_4H_4O_3(NH_2)_2 + H_2O + H_2 = C_4H_4O_4(NH_4)_2 .$$

---

[1] The solubility of asparagine in cold water is given very variously, the tements ranging from 1 in 12 to 1 in 300.

[2] Piutti (*Comp. rend.*, ciii. 134 ; *Ber.*, xix. 1691) obtained from 6500 logrammes of the shoots of the vetch about 20 kilogrammes of crude asparane, from which he isolated 100 grammes of a *dextro-asparagine*, having a ry sweet taste and exhibiting in aqueous solution a *dextro*-rotation of +5·5°. he compounds possessed a reverse rotation, but were otherwise exactly milar to those yielded by lævo-asparagine. Both modifications gave the me inactive aspartic acid when heated under pressure with hydrochloric id.

A similar change results when asparagine is taken internally, the urine after asparagus has been eaten acquiring a peculiar odour and containing ammonium succinate.

Asparagine exhibits both an acid and a basic function. The hydrochloride, $C_4H_8N_2O_3HCl$, forms large, readily soluble crystals. $Cu(C_4H_7N_2O_3)_2$, is obtained by treating a solution of asparagine with cupric hydroxide or cupric acetate. It resembles the corresponding compound of glycocine (page 209).

Asparagine reduces Fehling's solution on boiling, which reaction distinguishes it from glutamine.

When asparagine is dissolved in cold nitric acid (free from nitrous acid) it is converted into aspartic acid and ammonium nitrate; but if nitrous acid be present, or if nitric oxide or nitrous fumes be passed into the solution, the aspartic acid is converted into malic acid, with evolution of nitrogen, the reaction being, according to Sachsse and Kormann:—

$$C_4H_8N_2O_3 + 2HNO_2 = C_4H_6O_5 + 2H_2O + 2N_2 .$$

The most characteristic reaction of asparagine is its conversion into aspartic acid and ammonia by treatment with alkalies or mineral acids. The change readily occurs when asparagine is boiled with water and lime, baryta, or litharge; or with dilute hydrochloric or sulphuric acid:—

$$C_4H_8N_2O_3 + H_2O = C_4H_7NO_4 + NH_3 .$$

The reaction might possibly be made quantitative, but E. Schulze has shown that there is a tendency to further decomposition if the action be too prolonged. Boiled with water alone at the atmospheric pressure for twelve hours, only 2 per cent. of the asparagine was converted into ammonium aspartate. Under higher pressure the conversion was much greater. Milk of lime had no action in the cold after twenty-four hours, but on boiling with lime or baryta the action was much more rapid. When a large excess of baryta was used, the conversion was complete in one hour, but on continuing the treatment some hours more a further elimination of ammonia occurred, with formation of malic acid. Boiling with water containing one-tenth of its measure of strong hydrochloric acid effected complete conversion in one hour, action on the aspartic acid occurring if the treatment were further prolonged. Schulze also obtained good results by treating 2 grammes of asparagine with 5 c.c. (=8·79 grammes) of pure sulphuric acid and 100 c.c. of water, and boiling under a reflux condenser for two hours. The cooled liquid was nearly neutralised with soda and distilled with magnesia. The ammonia found in the distillate was fairly in accordance with theory. With a smaller quantity of acid, the results were less accurate, and the boiling had to be prolonged.

A method of determining asparagine in plant-products has been based on this reaction by R. Sachsse (*Jour. pract. Chem.*, [2], vi. 118); but for this purpose it is necessary previously to get rid of various co-occurring matters. Sachsse boils 10 grammes of the powdered substance for fifteen minutes with 200 c.c. of a mixture of equal volumes of alcohol and water, under a reflux condenser. 5 c.c. of a cold saturated solution of mercuric chloride[1] in alcohol is diluted with an equal measure of water and added to the decoction while still hot, and the liquid filtered, the residue being washed first with proof-spirit and then with cold water.[2] The filtrate is evaporated to dryness, the residue taken up in the minimum quantity of hot water (not more than 50 c.c.), and sulphuretted hydrogen passed through the filtered liquid, without filtering. The filtrate from the precipitated mercuric sulphide is then brought to a volume of 100 c.c., 10 c.c. of hydrochloric acid added, and the liquid boiled under a reflux condenser for one hour. The ammonia formed is then decomposed by alkaline hypobromite (Knop's method) and the evolved nitrogen measured;[3] or the liquid is neutralised by soda and distilled with magnesia, the ammonia in the distillate being determined by titration with standard acid. 17 parts of ammonia or 14 of nitrogen resulting from the treatment with hydrochloric acid correspond to 150 parts of crystallised asparagine originally present.

In employing the above process, a correction must be made for any ready-formed ammonia or other substances evolving nitrogen with the hypobromite reagent without treatment with hydrochloric acid (such as Sachsse found to exist in germinating peas). This is done by treating one-half of the filtrate from the mercuric sulphide precipitate with the hypobromite reagent direct (omitting the treatment with hydrochloric acid) and deducting any nitrogen evolved from the total amount before calculating it into asparagine.

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[1] Schulze (*Ber.*, xv. 2255) employs mercuric nitrate in place of mercuric chloride for the precipitation of asparagine, and for its separation from carbohydrates.

[2] The washing may be avoided by making up the liquid to 500 c.c., passing it through a dry filter, and evaporating 400 c.c. of the filtrate (=8 grammes of material).

[3] This method of determining asparagine has been investigated in the author's laboratory by A. R. Tankard. The asparagine was hydrolysed by heating with dilute hydrochloric acid for an hour and a half, in the manner directed by Sachsse, the liquid neutralised, and an aliquot part treated with the hypobromite reagent in the manner employed for determinations of urea. It was found that low results were obtained in the cold, but that if the reaction were completed by immersing the flask in boiling water for a few minutes, the volume of gas, measured after cooling, and corrected for pressure, &c., was about 49 per cent. of the total nitrogen, and hence only 1 per cent. less than the theoretical yield.

The asparagine determined as above will include any glutamine which may be present, and the hypobromite reagent also evolves nitrogen from certain other organic bodies, such as leucine, caffeine, &c. Estimations of asparagine by Sachsse's method in young lupines were found by Schulze and Barbieri to agree very nearly with the quantity obtained by crystallisation. Amido-compounds generally are, in their opinion, estimated with more certainty by the method of Sachsse and Kormann (*Zeits. anal. Chem.*, xiv. 380), in which the asparagine, &c., is first decomposed by boiling with dilute acid, and the resultant amido-acids, after removal of ammonium salts, treated with potassium nitrite and dilute sulphuric acid, the evolved nitrogen being measured. By this treatment all known amido-compounds are decomposed. Albuminoids and peptones must be removed before the amido-compounds can be determined. The following are the proportions of nitrogen in different forms found by Schulze and Barbieri in certain plant-products. The peptone-nitrogen, B, is that thrown down by phospho-tungstic acid in the filtrate from the albuminoids. D is the difference between the total nitrogen and that existing i the A, B, and C forms.

Substance.	Albuminoid Nitrogen. A.	Peptone Nitrogen. B.	Amido-Nitrogen. C.	Unknown Forms. D.	Total Nitrogen. E.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Lupine seeds, . .	8·17	0·24	...	...	8·63
Soja beans, . . .	6·32	0·13	...	...	6·73
11–12 days old sprouts of lupine, . .	3·40	1·60	...	...	10·64
12 days old sprouts of lupine, . .	2·33	2·17	5·59	0·42	10·61
15 days old sprouts of Soja beans, .	3·86	0·56	2·47	0·53	7·42
Birch leaves, . .	3·11	0·15	0·66	0·40	4·32
Young grass, . .	1·55	0·21	0·22	0·20	2·22

C. Böhmer (*Landw. Versuchs.—Stat.*, xxviii. 247 ; abst. *J Chem. Soc.*, 1883, 237) has given the following data respecti node of occurrence of nitrogen in various vegetables, which cut when fit for use. The figures are percentages, and refer ie moisture-free substances. The water ranged from 4·3 in the les to 96 per cent. in the asparagus.

Vegetable.	N as Albds.	N as Amido-acid Amide.	N as Amido-Acid.	N as Ammonia.	Total Nitrogen.
nach,	3·51	0·123	0·068	0·021	4·56
s,	3·56	0·052	0·361	0·020	4·69
ns,	4·39	0·027	0·059	0·013	5·57
aragus,	3·33	...	...	?	4·13
tuce,	2·97	0·155	0·154	0·024	4·85
rot,	1·57	0·013	0·142	0·006	1·91
nip-cabbage,	2·05	0·151	0·231	0·018	4·64
liflower,	2·60	0·104	0·566	0·017	5·11
nch beans,	2·67	0·061	0·442	0·010	4·32
shrooms,	3·34	0·092	0·416	0·011	4·68
ffles,	3·63	0·072	0·202	0·008	4·50

n the foregoing analyses, the ammonia was determined by milk me, in the manner recommended by Schloesing and modified Schulze and Emmerling, and weighed as chloroplatinate. To rmine the amido-acids and acid amides, the albuminoids were ipitated by Stützer's method with cupric hydroxide, and the ate concentrated and divided into three parts. One of these treated at once with hypobromite; the second was boiled for hours with hydrochloric acid, neutralised, and treated with bromite. The difference between the volumes of nitrogen ved in the first and second experiments represents the nitrogen ved from ammonia produced by the hydrolysis of the aspara- and glutamine. The third portion was boiled first with rochloric acid, and next with caustic alkali to volatilise the nonia. It was then treated with nitrous acid for the estimation he aspartic and other amido-acids, the nitric oxide, &c., evolved the nitrogen being absorbed by a strong solution of per- ganate.

or the isolation of asparagine and glutamine from vegetable s and extracts, E. Schulze (*Zeits. Anal. Chem.*, xxii. 325) ipitates the liquid with basic lead acetate. The filtered solu- is then treated with a neutral solution of mercuric nitrate t made by adding caustic soda to an acid solution until it no

longer reddens methyl-orange). The white flocculent prec
is filtered off, washed with cold water, and decomposed l
phuretted hydrogen. The filtered liquid, boiled to free it
sulphuretted hydrogen, will, if asparagine or glutamine be p
evolve ammonia when boiled with caustic alkali, and will d
cupric hydroxide to a deep blue solution. Allantoin i
precipitated by mercuric nitrate, but does not dissolve
hydroxide, and is precipitated on adding silver nitrat
ammonia. Xanthine, if present, will also be thrown do
the mercury. For the actual isolation of the amides, the
from the mercuric sulphide precipitate should be neutralise
ammonia and evaporated to a small bulk, when asparagi
glutamine will be deposited in crystals on cooling. (
original plant-juice, after boiling and filtering from coag
albumin, may be acidulated with sulphuric acid, and the pe
and ammonia precipitated by phospho-tungstic acid. After
ing two hours, the precipitate is filtered off, and the asp
and glutamine estimated in the filtrate by boiling with
hydrochloric acid, and determining the ammonia formed by
lation with magnesia or treatment with alkaline hypol
(page 221).

Other amido-compounds occur in plants, and are more
liable to be estimated as asparagine unless special mea
taken to separate them. E. Schulze, to whom the e
knowledge on the subject is largely due, finds the exact
of the amido-compounds to vary with the plant under ex
tion, and its age and conditions of life. In the *Caryoph*
and *Filices* asparagine is entirely replaced by its hom
glutamine.[1]

Aspartic Acid, $C_2H_3(NH_2)(COOH)_2$, has the constitutio
amidosuccinic acid. It occurs in beetroot molasses,
less as a product of the decomposition of asparagine, in spen

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[1] In *Lupinus luteus*, Schulze found asparagine, phenylalanine,
valeric acid, arginine, choline, and xanthine-like substances; in *C*
pepo, glutamine, asparagine, leucine, tyrosine, arginine, choline, vern
xanthine-like substances; in *Vicia sativa*, asparagine, phenylalanine,
amidovaleric acid, guanidine, choline, and betaïne. This does not
that in plant-metabolism the proteid molecule breaks down in differe
it being contended that the disintegrative metabolism of proteid i
tatively the same, but varies quantitatively. This view is supported b
ments on plants of the same kind, but of different ages. Schulze
that in some plants certain varieties of nitrogenous crystalline compo
used more in nourishing the tissues, whilst in other plants other con
are more advantageous, and so are used up first.

lees or vinasse, and in other vegetable juices.[1] It is also formed by boiling albumin or casein with dilute sulphuric acid, by the action of stannous chloride on horn, by treating proteids with bromine, &c.

Aspartic acid is best prepared by the hydrolysis of asparagine. H. Schiff (*Ber.*, xvii. 2929) recommends that 100 grammes of asparagine should be boiled for two or three hours under a reflux condenser with 408 c.c. of hydrochloric acid, containing 48·65 grammes of real HCl; that is, sufficient for the reaction:—

$$C_4H_8N_2O_3,H_2O+2HCl=C_4H_7NO_4,HCl+NH_4Cl.$$

To the cooled solution is added about 200 c.c. of ammonia, containing an amount of real NH_3 sufficient to neutralise just one-half the acid previously employed. (The other half has been neutralised by the ammonia formed in the hydrolysis.) On cooling the liquid and allowing it to stand, aspartic acid separates in colourless crystals.

Aspartic acid forms small rectangular plates, having a specific gravity of 1·66. It dissolves in about 360 parts of cold water, or in 19 of boiling water, and hence is much less soluble than asparagine. In alcohol it is nearly insoluble. The solutions of aspartic acid in alkalies are lævo-rotatory, while those in hydrochloric acid exhibit a dextro-rotation ($a_D=+28°$).[2]

Aspartic acid forms a series of crystallisable salts with bases. The *cupric salt*, $Cu,C_4H_5NO_4,4\frac{1}{2}$ aqua, forms blue needles, soluble in hot water, but very sparingly soluble in cold water (1 : 2800). This fact may be employed for the detection and isolation of aspartic acid, solutions of which may be precipitated by cupric acetate (see Lewinsky, *Chem. Centralb.*, 1894, i. 53).

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[1] For the isolation of aspartic acid, the boiling liquid containing it should be treated with carbonate of barium or lead, and alcohol added as long as further precipitation occurs. The precipitate is treated with water, and the barium or lead aspartate reprecipitated by addition of alcohol. The precipitate is again dissolved in water, the barium or lead precipitated with dilute sulphuric acid, and the filtered liquid evaporated to the crystallising point. The crystals are purified by treatment with 60 per cent. spirit, and the residue boiled with water, when pure aspartic acid crystallises out on cooling.

[2] Several optically inactive modifications of aspartic acid have been produced by synthetical means. By heating an aqueous solution of the hydrochloride of the active acid to 170°–180° for some hours, an inactive acid is formed which is identical with that obtained from the ammonium salts of malic, maleic, or fumaric acid. A lævo-rotatory aspartic acid has been prepared from dextro-asparagine by treatment with hydrochloric acid. Its properties are the same as those of ordinary aspartic acid, with which it combines to form an inactive acid (see *Comp. rend.*, cvi. 1734).

Aspartic acid reduces Fehling's solution.

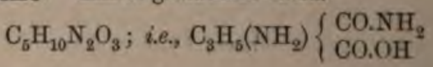
Aspartic acid is not decomposed by alkaline hypobromite solution, but by treatment with nitrous acid it is converted into malic acid with evolution of nitrogen :—

$$C_4H_7NO_4 + HNO_2 = C_4H_6O_5 + H_2O + N_2.$$

This reaction is employed by Sachsse and Kormann for the determination of aspartic acid, and, indirectly, of asparagine. In practice, sodium nitrite and dilute sulphuric acid are substituted for nitrous acid. Millon's reagent would probably be preferable.

F. Meunier (*Ann. Agronomiques*, vi. 275 ; *Jour. Chem. Soc.*, xl. 761) finds that the determination of asparagine by measurement of the nitrogen evolved by the action of nitrous acid is inaccurate. He has devised the following process, which depends upon the production of potassium aspartate and ammonia, when asparagine is treated with potassium hydroxide. The crushed, dried, and weighed substance is placed in a little bag with meshes small enough to retain the starch. This is placed in a porcelain dish, exhausted with boiling water, the filtered solution is heated with subacetate of lead to precipitate albuminoids and leucine, and the excess of lead is removed from the filtrate by sodium hydrogen carbonate. The filtrate from the lead carbonate is distilled with caustic alkali, and the ammonia in the distillate titrated with standard acid. When ammonium salts are present, they must be separately estimated.

Glutamine. Amido-glutaminic Acid.



$$C_5H_{10}N_2O_3 ;\ i.e.,\ C_3H_5(NH_2)\left\{\begin{array}{l}CO.NH_2\\CO.OH\end{array}\right.$$

Glutamine is the higher homologue of asparagine, and co-occ with it in beetroot, pumpkins, and the shoots of vetch. In t families *Caryophyllaceæ* and *Filices* glutamine completely repla asparagine. Glutamine is also a product of the action of dil acids or baryta on proteids.

Glutamine forms slender anhydrous needles, soluble in 25 pa of cold water, and much more readily at the boiling-point. It insoluble in absolute alcohol. The aqueous solution (4 gram per 100 c.c.) is optically inactive, but the solutions in hyd chloric and oxalic acids are dextro-rotatory.

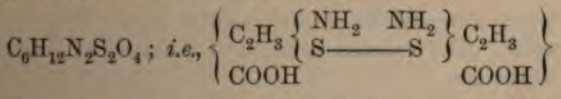
When heated with alkalies or dilute mineral acids, glutami yields ammonia and glutamic acid, a body homologous wi aspartic acid (page 224).

Glutamine does not reduce Fehling's solution, but dissolv cupric hydroxide to a deep blue solution, a crystallisable compou being formed analogous to that yielded by glycocine (page 209). Glutamine forms an insoluble compound with mercuric nitrate, a fact utilised by Schulze and Bosshard to isolate it from the juice of beetroot (*Ber.*, xvi. 312; xviii. 290). (See page 224.) Glutamine, asparagine, and other amido-compounds are not precipitated by an acid solution of phospho-tungstic acid.

Glutamic Acid, $C_3H_5(NH_2)(COOH)_2$, is the higher homologue of aspartic acid, bearing the same relation to normal pyrotartaric acid that aspartic acid bears to succinic acid. Glutamic acid has been isolated from molasses after the sugar has been removed by the strontia process, and is formed, together with aspartic acid, by boiling vegetable proteids (*e.g.*, conglutin, maize-fibrin) with dilute sulphuric acid.

Glutamic acid forms trimetric tetrahedra, melting at 202°. It dissolves in about 100 parts of cold water, and is less soluble in spirit (1:500). The solutions are acid, and have an astringent taste. The aqueous solution of glutamic acid and of its hydrochloride are dextro-rotatory, but its salts with bases are lævo-rotatory. Glutamic acid differs from aspartic acid in yielding no precipitate with lead acetate even after the addition of ammonia; but the lead salt may be precipitated by adding alcohol to the concentrated filtrate from any precipitate produced by basic lead acetate. Glutamic acid is also distinguished from aspartic acid by not reducing Fehling's copper solution on heating. It forms a characteristic copper salt which is very sparingly soluble in cold water. This fact may be used for the isolation of glutamic acid.

Cystin. Dithio-diamido-lactic Acid.



$$C_6H_{12}N_2S_2O_4;\ i.e., \left\{\begin{matrix} C_2H_3\left\{\begin{matrix}NH_2 & NH_2\\ S\text{———} & S\end{matrix}\right\}C_2H_3 \\ COOH \qquad\qquad\qquad COOH\end{matrix}\right\}$$

Cystin is the leading constituent of rarely-occurring urinary and renal calculi. It is also met with as a sediment from urine. It may be prepared from such sediment, or preferably from the calculus when obtainable, by treating the substance with ammonia, and allowing the filtered liquid to evaporate spontaneously, when the cystin is deposited in characteristic colourless or pale yellow hexagonal tables of sharp contour (fig. 5), which are often superposed.

Cystin is colourless, odourless, and tasteless. When heated, it ignites without melting, and burns with a greenish-blue flame, emitting a characteristic penetrating odour resembling that of hydrocyanic acid. Heated in a closed tube, cystin gives off ammonia and yields a distillate of disagreeable odour, leaving a residue of carbon.

Cystin is neutral to litmus, and insoluble in water, alcohol, and ether. It is readily soluble in ammonia (distinction from uric acid), in fixed caustic alkalies and alkaline carbonates, but not in ammonium carbonate. It is precipitated from these solutions by acetic acid. Cystin dissolves in mineral acids and in oxalic acid, but not in tartaric or acetic acid. The solution in hydrochloric acid containing 11·2 per cent. of HCl has a specific rotation of $[\alpha]_D = -206°$.[1]

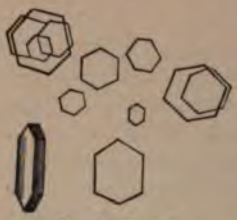


Fig. 5.—Cystin.

Cystin forms unstable salts with acids, and is precipitated from their solutions by ammonium carbonate. The hydrochloride unites with mercuric chloride to form a crystalline compound which is nearly insoluble in water. P. Borissaw has attempted to utilise this reaction for the determination of cystin in urine (*Zeits. physiol. Chem.*, xix. 511).

If a cold solution of cystin in ammonia be treated with ammonio-nitrate of silver, and the liquid then cautiously neutralised by nitric acid, a canary-yellow precipitate is thrown down, but if the solution be heated silver sulphide is precipitated.

When treated with granulated tin in hydrochloric acid solution, cystin is reduced to amidothiolactic acid or cystein, $C_3H_7NSO_2$:—

$$COOH.C_2H_3(NH_2)S-S(NH_2)C_2H_3.COOH+H_2=\\ 2[HS(NH_2)C_2H_3.COOH].$$

Cysteïn is a crystalline powder, soluble in water, in ammonia, and in acids. The aqueous solution is oxidised to cystin on exposure to the air.

By reaction with nitrous acid cystin yields pyruvic acid, $CH_3.CO.COOH$.

When heated with nitric acid, cystin is decomposed with production of a brown coloration.

When boiled with caustic alkalies, cystin evolves ammonia. The solution then contains a sulphide, and hence gives a black precipitate on addition of lead acetate. The sulphur is not wholly converted into sulphide, even after many hours boiling with caustic alkali.

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[1] E. Kulz (*Ber.*, xv. 1410) gives −142° as the specific rotation of cystin for the transition-tint in ammoniacal solution.

For the detection of cystin in a calculus, the powdered substance should be dissolved in caustic alkali, and acetic acid added to the hot solution, when cystin, if present, will separate on cooling, and can be recognised by its crystalline form. Or the calculus may be treated with hot ammonia, and the filtered liquid evaporated to the crystallising point. Any xanthine will be dissolved out and deposited with the cystin.

Goldmann and Baumann (*Zeits. physiol. Chem.*, 1888, 254) contradict the statement of Stadthagen that normal urine contains little or no cystin, as by the following process they have proved that cystin or an allied substance is always present in urine. This method is based on the fact that when a few drops of benzoyl chloride are added to a solution of cystin in caustic soda, a voluminous precipitate of shining plates of the sodium salt of benzoyl-cystin, $C_6H_{10}N_2S_2O_4Bz_2$, is formed. This compound is soluble in hot water, less soluble in cold, and quite insoluble when excess of caustic soda is present. On adding a strong acid to the dilute solution, the liquid sets to a transparent jelly, but on warming and standing free benzoyl-cystin separates in dense flocks which can be separated by filtration. Benzoyl-cystin is a strong acid, almost insoluble in water, and but slightly soluble in pure ether, but more readily in ether containing alcohol. In alcohol it dissolves, and crystallises from the solution in slender needles which tend to aggregate in cauliflower-like masses. Benzoyl-cystin melts at 156°–158°. By heating with strong hydrochloric acid, it is decomposed into benzoic acid and cystin. When boiled with caustic soda and lead acetate, it yields black lead sulphide, but the decomposition is not complete even after prolonged boiling.

For the isolation of cystin from urine, Goldmann and Baumann recommend that 200 c.c. of the sample should be treated with 10 c.c. of benzoyl chloride and 70 c.c. of caustic soda solution of 1·12 specific gravity, and the mixture shaken until the benzoyl chloride has dissolved. The precipitate (which consists of benzoyl compounds of urinary carbohydrates, mixed with phosphates) is filtered off, and the filtrate rendered strongly acid with sulphuric acid, and shaken with ether containing alcohol. The ethereal layer is separated, evaporated, and the residue boiled for one hour with caustic soda and lead acetate. The lead sulphide produced is equivalent to about two-thirds of the cystin isolated; the cystin represented being three-fourths of the actual weight of PbS obtained. From 200 c.c. of normal urine, Goldmann and Baumann obtained 0·0025 gramme of lead sulphide, representing 0·0009 of cystin, for 100 c.c. of urine.

According to J. L. W. Thudichum, the colouring matter of urine, which he calls urochrome, is completely precipitated by treating urine with benzoyl chloride and soda as above described. The precipitate is soluble in alcohol, and may be purified by boiling with water. Glucose and certain diamines (page 334) occasionally present in urine are also precipitated by benzoyl chloride. The amount of cystin in the mixed benzoyl compounds might be deduced from a determination of the sulphur.

Taurin. Amidoethane-Sulphonic Acid.

$C_2H_7NSO_3$; or $[CH_2(NH_2).CH_2(SO_2.OH)]$

This interesting substance is frequently regarded as identical with amido-isethionic acid, with which compound it is in fact isomeric.

Minute quantities of taurin are stated to exist in the juices of the lungs and of muscles, but its principal mode of occurrence is in the form of taurocholic acid, $C_{26}H_{45}NSO_7$, which is a characteristic constituent of the bile of the dog and other carnivora.[1] (See Bile Acids.)

Taurin is best prepared by boiling ox-bile for some hours with dilute hydrochloric acid, separating the liquid from the resinous product, and precipitating the remaining traces of bile-acids by lead acetate. The filtrate is freed from lead by sulphuretted hydrogen, concentrated, and the taurin which separates on cooling purified by recrystallisation from water.

Taurin has been obtained synthetically by the following series of reactions:—Ethylene, C_2H_4, is absorbed by fuming sulphuric acid, the product dissolved in water, neutralised by ammonia, and the solution evaporated to the crystallising point. The resultant ammonium isethionate, $C_2H_4O.SO_3(NH_4)$, when heated to 220° C. yields taurin and water. Taurin has also been obtained by con-g ethylene into glycol-chlorhydrin, and treating that body in llowing manner :—

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[1] Free taurin was found by Gorup-Besanez in the liver of a person who died from *arachnitis*. It has been detected in the liver in cases of jaundice, and has also been met with in the kidneys, lungs, and muscles. It is likewise present in the intestinal canal and in excrement, doubtless as a product of the decomposition of taurocholic acid.

The taurin of the bile undergoes change in the alimentary canal of man, and appears in the urine chiefly in the form of tauro-carbamic acid, $NH_2CO.NH(CH_2).CH_2.(SO_2OH)$. In dogs, some tauro-carbamic acid is formed, but a large proportion is excreted unaltered; while in rabbits, on the other hand, some taurin is excreted unchanged, but the greater portion is oxidised, so that the urine contains a greatly increased proportion of sulphates, together with some thiosulphates (hyposulphites). When injected hypodermically, taurin is chiefly excreted unchanged.

l-chlorhydrin, . . $HO.C_2H_4.Cl$, heated with K_2SO_3, gives
sium isethionate, . $HO.C_2H_4.SO_2.OK$; which, distilled
h PCl_5, yields
onic chloride, . . $Cl.C_2H_4.SO_2.Cl$; which, on heating
h H_2O, yields
ethyl-sulphonic acid, $Cl.C_2H_4.SO_2.OH$; which, with NH_3 at
)° under pressure, gives
n, ... $NH_2.C_2H_4.SO_2.OH$

urin crystallises in hard, six-sided prisms (fig. 6), which le between the teeth. It melts at 240° C. with intumescence volution of sulphur dioxide, &c., leaving a difficultly combie carbonaceous residue.

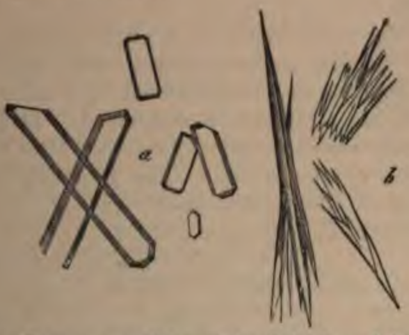

Fig. 6.—TAURIN. *a*, well-formed six-sided prisms; *b*, irregular sheaf-like masses from an impure solution.

urin has a fresh taste, and is readily soluble in water, but is sparingly soluble in rectified spirit (1:500), and is practically ible in absolute alcohol and ether.

urin has no acid reaction, but it forms soluble crystallisable with bases. It dissolves in hot dilute acids, and separates nged on cooling. When the solution of taurin is evaporated caustic alkali, the whole of the nitrogen is evolved as am-, and the residue contains a sulphite and acetate of -metal. Fused with caustic potash, the same products are ned. If heated strongly with sodium carbonate, avoiding of air, taurin yields a product containing much sodium ide. Hence the solution of the mass in water blackens a coin, and evolves sulphuretted hydrogen when treated with id.

By reaction with nitrous acid, taurin yields isethionic acid.

Solutions of taurin are not precipitated by metallic salts or by tannin.

For the detection of taurin or taurocholic acid in bile, the liquid should be kept till it acquires a strongly acid reaction. It is then treated with acetic acid, the filtered liquid evaporated at 100°, and the residue extracted with absolute alcohol, when the taurin will be left undissolved. For the detection of taurin in excrement, &c., the substance should be thoroughly dried, exhausted with cold water, the filtered solution evaporated, and the residue treated with absolute alcohol, as described above (compare page 396).

BETAÏNES.

On page 209, it was stated that glycocine was the starting-point of two distinct series of bases. One series, having the characters of amido-acids, is represented by leucine, tyrosine, and asparagine, and has already been considered. The bases of the second series are called generically *betaïnes*, after the name of a typical member of the group. The following are the most important bases of the betaïne class:—

		Base.	*Salt.*
Glycocine (page 206),	$C_2H_5NO_2$,	$N\left\{\begin{array}{l}H_3\\ \left.\begin{array}{l}CH_2.CO\\ O \;—\end{array}\right\}\end{array}\right.$	$N\left\{\begin{array}{l}H_3\\ CH_2.COH\\ Cl\end{array}\right.$
Sarcosine (page 223), (Methyl-glycocine.)	$C_3H_7NO_2$,	$N\left\{\begin{array}{l}(CH_3)H_2\\ \left.\begin{array}{l}CH_2.CO\\ O \;—\end{array}\right\}\end{array}\right.$	$N\left\{\begin{array}{l}(CH_3)H_2\\ CH_2.COH\\ Cl\end{array}\right.$
Betaïne (page 234), (Trimethyl-glycocine.) (Dimethyl-sarcosine.)	$C_5H_{11}NO_2$,	$N\left\{\begin{array}{l}(CH_3)_3\\ \left.\begin{array}{l}CH_2.CO\\ O \;—\end{array}\right\}\end{array}\right.$	$N\left\{\begin{array}{l}(CH_3)_3\\ CH_2.COH\\ Cl\end{array}\right.$
Neurine (page 236),	$C_5H_{13}NO$	$N\left\{\begin{array}{l}(CH_3)_3\\ CH{:}CH_2\\ OH\end{array}\right.$	$N\left\{\begin{array}{l}(CH_3)_3\\ CH{:}CH_2\\ Cl\end{array}\right.$
Choline (page 238),	$C_5H_{15}NO_2$,	$N\left\{\begin{array}{l}(CH_3)_3\\ CH_2.CH_2(OH)\\ OH\end{array}\right.$	$N\left\{\begin{array}{l}(CH_3)_3\\ CH_2.CH_2(OH)\\ Cl\end{array}\right.$
Muscarine (page 245), (Hydroxy-choline.)	$C_5H_{15}NO_3$,	$N\left\{\begin{array}{l}(CH_3)_3\\ CH_2.CH(OH)_2\\ OH\end{array}\right.$	$N\left\{\begin{array}{l}(CH_3)_3\\ CH_2.CH(OH)_2\\ Cl\end{array}\right.$
Isomuscarine (page 246),	$C_5H_{15}NO_3$,	$N\left\{\begin{array}{l}(CH_3)_3\\ CH(OH).CH_2(OH)\\ OH\end{array}\right.$	..

It will be seen that glycocine, sarcosine, and betaïne may be regarded as internal anhydrides, thus differing by the elements water from the remaining members of the group, which have constitution of hydroxides of ammonium-bases.

Ernst Schmidt considers a molecule of water essential betaïne, which he expresses by the formula $C_5H_{13}NO_3$, or $H_3)_3N(OH).CH_2.COOH$. Muscarine is an intermediate proct of the oxidation of choline, and Schmidt suggests that the ee bases, choline, muscarine and betaïne bear the same relation each other as alcohol, aldehyde, and acetic acid; but a comrison of their constitutional formulæ shows that this conjecture not warranted by the facts.

Schmidt further suggests that, if muscarine have an aldehydic stitution, its toxic character is probably due to presence of the ehyde group $-CH_2.CH(OH)_2$, or of the group $-CH_2.COH$; ce the groups $-CH_2.CH_2.OH$ and $-CH_2.COOH$, in combina- with trimethylamine, have no direct poisonous action. In case of neurine, on the contrary, it is probable that the toxic on may be connected with the double linking in the vinylup $-CH{:}CH_2$. Hence it might be expected that a corrending trimethylamine derivative with a triple linking would e a similar or even stronger toxic action. This inference been found correct, acetenyl-trimethylammonium hydroxide, $I_3)_3.(OH).C{:}CH$, being a more powerful poison even than neurine. the other hand, allyl-trimethylammonium hydroxide, which the constitution of a higher homologue of neurine, is a comtively non-poisonous substance.

t is interesting to observe that while muscarine is intensely onous the isomeric base isomuscarine is relatively inert. ce the toxic character of the former substance appears to end upon the existence of two loosely-combined hydroxyltps attached to the same carbon-atom. This conjecture opens ide field for future experiment.

ll the above bases belong to the class of ptomaïnes, and a ber of ptomaïnes of unknown constitution are not improbably bers of the betaïne-group (compare page 343).

arcosine. Methyl-glycocine.

$$C_3H_7NO_2{}^1;\ i.e.,\ N^{v}\left\{\begin{array}{l}(CH_3)H_2\\ CH_2.CO\\ O\end{array}\right.;\ \text{or}\ N\left\{\begin{array}{l}(CH_3)H\\ \\ CH_2.COOH\end{array}\right.$$

arcosine is prepared by boiling creatine with an aqueous solution

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Sarcosine is isomeric with alanine, lactamide, and urethane. It is disuished from these bodies by its insolubility in alcohol and ether, in tion, of course, to various chemical reactions.

of ten times its weight of baryta, until all odour of ammonia has disappeared. The creatine is decomposed into sarcosine and urea, the latter product being further split up into ammonia and carbonic acid. The excess of baryta is removed by a current of carbon dioxide, the liquid boiled, filtered, and evaporated to a syrup, from which the sarcosine is deposited in foliated crystals on standing. Sarcosine also results from the action of acids or alkalies on caffeine and theobromine (Part ii. pages 478, 494); and W. Paulmann (*Arch. Pharm.*, ccxxxii. 601) recommends the hydrolysis of caffeine as the best method for the preparation of sarcosine, the yield being 60 per cent. of the theory.

Sarcosine has been obtained synthetically by digesting ethyl chloracetate under pressure, at 125°, with an excess of a concentrated solution of methylamine :—$CH_2Cl.COO(C_2H_5)+NH_2Me+H_2O=NHMe.CH_2.COOH+C_2H_5.OH+HCl$. The yield by this method is very poor.

Sarcosine may be purified by conversion into the sulphate, the aqueous solution of which is then decomposed by pure barium carbonate.

Sarcosine crystallises in colourless, transparent, rhombic prisms, having a sharp, sweetish, somewhat metallic taste. Sarcosine is unchanged at 100°, but at a higher temperature melts and volatilises without leaving any residue.

Sarcosine is readily soluble in water, sparingly soluble in alcohol, and insoluble in ether. It has no action on litmus, but combines with acids to form soluble crystallisable salts. B_2,H_2SO_4+2 aqua forms colourless, quadrangular crystals, very readily soluble in water. B_2,H_2PtCl_6+2 aqua is soluble in water, and crystallises in large, pale yellow, flattened octahedra. Sarcosine also reacts with bases. The *cupric salt*, $Cu(C_3H_6NO_2)_2+2$ aqua, forms deep blue crystals.

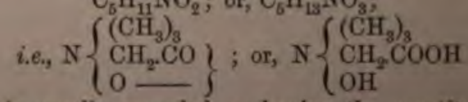
When heated with soda-lime, sarcosine evolves methylamine.

Benzoyl-sarcosine (methyl-hippuric acid), NMeBz.CH.COOH, has been prepared, but owing to its extreme solubility has not been obtained in crystals.

In all its chemical relationships sarcosine presents a close resemblance to glycocine.

Betaïne. Dimethyl-sarcosine. Oxycholine. Lycine.

$C_5H_{11}NO_2$; or, $C_5H_{13}NO_3$;



$$i.e.,\ N\left\{\begin{matrix}(CH_3)_3\\ CH_2.CO\\ O\ \text{——}\end{matrix}\right\}\ ;\ \text{or,}\ N\left\{\begin{matrix}(CH_3)_3\\ CH_2.COOH\\ OH\end{matrix}\right.$$

Betaïne is usually regarded as having the constitution of an internal anhydride, but E. Schmidt considers a molecule of water essential to its constitution and expresses it by the last of the above formulæ.[1]

Betaïne occurs naturally in the juice of beetroot (*Beta vulgaris*). The unripe root contains 0·25, but the ripe root only 0·10 per cent. The betaïne is not present in the root as such, but in a form from which it may be liberated by treatment with hydrochloric acid or baryta. Hence the compound is probably allied to the lecithins (page 241). Betaïne is also present in beetroot molasses, in the branches and leaves of *Lycium barbarum* (whence its name lycine), in mangold-wurzel, in cotton-seed, &c., and is a product of the decomposition of proteids.

For the preparation of betaïne, beetroot juice or molasses should be diluted with water and treated with lead acetate in slight excess. The precipitate, which contains the betaïne, is decomposed by dilute sulphuric acid, and the solution precipitated by phosphotungstic acid. The resultant precipitate gives free betaïne when treated with milk of lime.

Another plan is to boil the diluted molasses or beet-juice with baryta for twelve hours, filter, pass carbon dioxide, evaporate the filtered liquid to a syrup, and exhaust with alcohol. The extract is treated by an alcoholic solution of zinc chloride, the precipitate separated, recrystallised from water, and decomposed by baryta. The solution is exactly decomposed by dilute sulphuric acid, and the filtered liquid evaporated till the betaïne hydrochloride crystallises out.[2]

Betaïne has also been obtained by the oxidation of choline, by the action of chloracetic acid on trimethylamine, and by treating a caustic potash solution of glycocine with methyl iodide and methyl alcohol.

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[1] It is difficult to describe the betaïne of Schmidt's formula by a systematic name. Hydroxy-trimethyl-ammonium acetate is perhaps the best.

[2] Natural betaïne is the type of a series of similar bases obtainable synthetically. These may be prepared with but slight admixture of secondary products by the action of alkyl iodides on zinc salts of the amido-acids in presence of zinc oxide (E. Duvillier, *Compt. rend.*, cx. 640, and *Jour. Chem. Soc.*, lviii. 747).

A phosphorus-betaïne has been obtained artificially by A. W. Hofmann (*Proc. Royal Soc.*, xi. 525); and a curious series of bases called *thetines*, which may be regarded as betaïnes in which sulphur takes the place of nitrogen, have been described by Crum-Brown and Letts (*Trans. Royal Soc. Edin.*, 1878, page 571) *e.g.*:—

$$N^{v}\left\{\begin{array}{l}(CH_3)_3\\ CH_2.COOH\\ Br\end{array}\right. \qquad\qquad S^{iv}\left\{\begin{array}{l}(CH_3)_2\\ CH_2.COOH\\ Br\end{array}\right.$$

Betaïne hydrobromide. Thetine hydrobromide.

Betaïne crystallises from alcohol in large deliquescent crystals containing 1 aqua, which is lost at 100°, or by exposure over strong sulphuric acid. It is precipitated in scales on adding ether to its alcoholic solution. Betaïne is optically inactive, has a sweet taste, is not poisonous, and is neutral to litmus. When heated, it decomposes with evolution of trimethylamine, and gives an odour of burnt sugar.

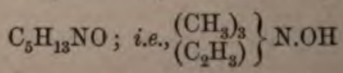
Betaïne is not affected by chromic or hydriodic acid; but on boiling or fusion with caustic alkali it yields trimethylamine.

The *salts* of betaïne may be regarded either as being formed by the replacement of the hydroxyl-group in Schmidt's formula by chlorous radicals, or as direct compounds of $C_5H_{11}NO_2$ with acids. Thus, the compound $C_5H_{12}NO_2Cl$ is either betaïne hydrochloride, $C_5H_{11}NO_2HCl$, or the chloride of the radical $C_5H_{12}NO_2$. It forms large, stable, monoclinic tables, melting with intumescence at 228° C., and very readily soluble in water, but nearly insoluble in absolute alcohol (distinction from choline). $(C_5H_{12}NO_2)_2PtCl_6$ forms large, yellow, efflorescent crystals, deposited from water in hexagonal plates, and from dilute alcohol in hydrated octahedra. The water of crystallisation is variously stated at 2, 3, and 4 molecules. The gold salt crystallises in thin needles or plates resembling cholesterin, soluble in water and melting at 209°. $B,ZnCl_2$ is crystallisable, soluble in water, but insoluble in strong alcohol.

With Mayer's reagent, a solution of betaïne hydrochloride yields a whitish-yellow precipitate, soluble in excess; but if the sides of the glass containing the precipitate be rubbed with a glass rod, yellow needles are deposited.

A solution of iodised potassium iodide precipitates betaïne as a periodide in brown crystals.

Neurine. Vinyl-trimethylammonium Hydroxide.



$$C_5H_{13}NO;\ i.e., \left.\begin{matrix}(CH_3)_3\\(C_2H_3)\end{matrix}\right\}N.OH$$

This base, discovered by A. W. Hofmann, differs from choline (page 237) by the elements of water. It occurs with choline in various animal substances and the products of their decomposition. According to Liebriech, choline is only formed when an alcoholic or ethereal extract of brain is operated on; while if free protagon be boiled with baryta-water, neurine is obtained instead.

Free neurine is only known in aqueous solution. It has alkaline reaction, and absorbs carbon dioxide from the

ine can be converted into neurine by heating it to 140° C.
ming hydriodic acid, and eliminating the iodine from the
t by moist silver oxide. The reverse reaction has also been
l by heating neurine chloride with hydriodic acid, and then
the product with silver nitrate in aqueous solution.

rine and choline present very close resemblances, and
the few distinctions between them are important. Thus
chloride gives an abundant precipitate with tannin, while
oline salt is not affected. On the other hand, choline
e is precipitated by phospho-tungstic acid, which with
gives no reaction. Choline platinichloride forms large,
red, tabular, monoclinic crystals, arranged like steps.
melt in a capillary tube at 232° to 233°, but generally at
o 241° with much frothing. The platinum salt of neurine
s $(C_5H_{12}NCl)_2,PtCl_4$, and crystallises in small, individual,
-red, regular octahedra, which melt at 211° to 213°, and
e with difficulty in hot water. The crystals soon turn
and on re-treatment with water leave an insoluble residue,
the platinum salt of choline is found in the solution.

Marino-Zuco (*Gazetta Ital.*, xiii. 431) has pointed out
eurine chloride is not decomposed by sodium bicarbonate,
ch it differs from the hydrochlorides of most of the poisonous
ble alkaloids. Hence if the mixed alkaloids and ptomaïnes,
aneously extracted by Stas' process, be dissolved in hydro-
acid, and the solution treated with sodium bicarbonate,
getable alkaloids can be extracted by agitation with ether,
form, amylic alcohol, &c., while the neurine and other
ptomaïnes remain in the aqueous liquid.

rine is extremely poisonous, the symptoms produced resem-
those due to poisoning by muscarine. Administered to a
bcutaneously, it soon produces paralysis of the extremities,
is followed by stoppage of the respiration, and finally of the
(in diastole). In rabbits, neurine occasions profuse nasal
on, salivation, and paralysis. Neurine produces contraction
pupil, both when injected and when applied locally. Atro-
as been found to be an efficient antidote, and even produces
rary immunity to poisoning by neurine. Hydroxy-trimethyl-
nium compounds are stated by V. Cervello to act similarly.

der the name of "cancroin," an aqueous solution of neurine,
ntaining phenol and citric acid, has been recommended by
nkiewicz as a hypodermic injection for the treatment of
(*Pharm. Jour.*, [3], xxiii. 606).

Choline. Hydroxyethyl-trimethylammonium Hydroxide.

$$C_5H_{15}NO_2\text{; } i.e., \left.\begin{matrix}(CH_3)_3\\(C_2H_4.OH)\end{matrix}\right\}N.OH$$

Choline has been prepared synthetically by treating ethylene oxide with a concentrated solution of trimethylamine.

Choline is a decomposition-product of lecithine, but also exists ready-formed in the tissues of living animals and plants, and is one of the first and most constant products of the putrefactive decomposition of proteids (compare page 235). It was first isolated from bile (whence the names choline and bilineurine), has been found in herring brine, and exists also in the brain and in yolk of egg, in the conjugate form of lecithine.

Choline may be classed both as a leucomaïne and a ptomaïne (page 192), being formed during normal vital actions as well as in putrefactive decomposition. It is the constant associate of neuridine during the earlier stages of putrefaction, being afterwards replaced by trimethylamine, which is no doubt produced by the decomposition of choline itself, $C_5H_{15}NO_2 = C_2H_6O_2 + C_3H_9N$. Choline may be isolated from putrefying matters by adding picric acid to the mother-liquor from which neuridine has been separated.

Besides occurring naturally in the animal kingdom, choline exists in a large number of plants and plant products. It has been shown to be identical with the base sincaline, obtained by the decomposition of sinapine occurring in white mustard seeds. It has also been found in many fungi, in germinated pumpkin sprouts, in the seeds of cotton (see page 240), *Vicia sativa* (vetch), *Trigonella Fœnum græcum* (fenugreek), ergot of rye, areca nuts, the fly agaric, ipecacuanha and hops; and was extracted by P. Griess from beer, which, according to J. Kjeldahl, also contains a choline derivative (lecithin ?).[1]

Choline may be prepared from yolk of egg by exhausting the substance with ether and afterwards with warm alcohol. These are distilled from the extract, the residue boiled for an hour with baryta-water, the excess of baryta precipitated by carbon dioxide, and the filtrate evaporated. The residue is exhausted with absolute alcohol, and the solution precipitated by platinic chloride. The platinichloride is dissolved in water, and decomposed by sulphuretted hydrogen. The solution of choline chloride is concentrated, and treated with silver oxide, when a strongly alkaline solution of free choline is obtained, and on evaporation the base remains as a syrupy liquid.

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[1] Kjeldahl found that the proportion of choline in beer was the same as that in the wort prior to fermentation. To extract it, he evaporates the beer or wort to one-half, and treats it with excess of milk of lime and one or two volumes of alcohol. The filtrate is acidified with sulphuric acid, evaporated on the water-bath with excess of barium carbonate till the alcohol is volatilised, and a large excess of iodised potassium iodide added. Needles of choline polyiodide, exhibiting a beetle-green reflection, are gradually deposited. These are exactly decomposed by sulphurous acid, the solution shaken with silver chloride (to convert the iodide into chloride), and the filtered liquid treated with platinic chloride to obtain the choline as a platinum salt.

Free choline is a deliquescent substance very difficult to crystallise. It usually forms a syrup. It is a powerful base, having an alkaline reaction, and absorbing carbon dioxide from the air.

By some observers (Arndt, &c.) choline is alleged to be volatile, but there is good reason to doubt the accuracy of this observation.

Choline, having the constitution of a tetrammonium hydroxide (compare Part ii. page 19), forms salts by the replacement of the OH-group by Cl, I, SO_4, &c. Thus the *chloride* has the formula $(C_2H_4OH)(CH_3)_3N.Cl$, and crystallises from absolute alcohol in fine deliquescent needles, readily soluble in alcohol and water (distinction from betaïne). The *platinum salt* has the composition $[(C_2H_4OH)(CH_3)_3NCl]_2,PtCl_4$, and crystallises from hot water in fine reddish-yellow plates or prisms, insoluble in absolute alcohol (compare page 237). The *gold salt* (Au = 44·5 per cent.) is deposited from a hot saturated aqueous solution in long yellow prisms, which melt at 244° to 245° C., and are soluble with difficulty in cold water, but dissolved by hot water or alcohol. *Choline sulphate* is amorphous, and almost insoluble in absolute alcohol, but very soluble in water. The *carbonate* is amorphous, very deliquescent, alkaline in reaction, and soluble in alcohol.

When choline is treated with hydriodic acid, both the hydroxyl-groups are replaced with formation of the body $(C_2H_4I)(CH_3)_3NI$; and when this is treated with moist oxide of silver and water (compare Part ii. page 20) it yields the base neurine, $(C_2H_3)(CH_3)_3N.OH$. (See page 237.) On oxidation, choline is converted into oxycholine, $(CH_2.COOH)(CH_3)_3N.OH$.

According to some observers, choline is not poisonous, but M. de Thierry states that it produces toxic symptoms similar to those of neurine, but less violent.[1]

Owing to the poisonous character of choline, W. Maxwell (*Amer. Chem. Jour.*, 1891, xiii. 469) has endeavoured to find out whether choline and betaïne are present in the cotton seed from which various cattle foods are prepared. Betaïne is generally believed to be non-poisonous, but is usually found together with choline.

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[1] Brieger found a dose of 0·005 gramme of choline chloride requisite to cause death to a rabbit, while one-tenth of that amount of neurine chloride proved fatal.

About 5 lbs. of finely-ground cotton-seed cake was extracted with 70 per cent. alcohol, the extract distilled, and the residue taken up in water. On adding lead acetate to this solution, a precipitate was thrown down which was separated, and the filtrate evaporated to a syrup after the excess of lead had been removed. The alkaloidal bodies were then taken up from this syrup in a mixture of 70 per cent. alcohol and 1 per cent. hydrochloric acid. This extract was treated with an alcoholic solution of mercuric chloride, when immediately an almost pure white double salt of the nitrogenous bases began to separate out. After standing for ten days the crystals were separated from the liquid, from which more crystals were deposited after some weeks. After recrystallisation from water the salt was decomposed by means of hydrogen sulphide. The filtrate, containing the chlorides of the bases, was slowly evaporated, and then placed in a desiccator over sulphuric acid until crystallisation of the salts was complete. The crystals, which were free from colour and well-developed, after drying, were saturated with absolute alcohol, in which the choline salt dissolved along with a small proportion of the betaïne salt. 7·248 grms. of the crystals were treated with alcohol, and the extract evaporated to dryness and re-extracted three times to obtain the choline salt free from betaïne. There were thus obtained 1·08 grm. of choline chloride, and 6·168 grms. of the corresponding betaïne salt. Choline and betaïne appeared to be present in the sample of cattle-food used by Maxwell in the relative proportions of 17·5 per cent. choline to 82·5 per cent. betaïne.

The alcoholic solution of the choline salt was treated with platinic chloride, and choline platinichloride obtained. From this an aqueous solution of choline chloride was obtained by treatment with hydrogen sulphide, and the separation of the resulting platinic sulphide by filtration. This aqueous solution gave the following reactions:—

With Phospho-tungstic acid, white precipitate.
Phospho-molybdic acid, yellow precipitate.
Bismuth-potassium iodide, red precipitate.
Cadmium-potassium iodide, grey precipitate.
Iodine, brown precipitate.
Platinic chloride, yellow precipitate soluble in water.

An aqueous solution of the betaïne hydrochloride was treated with phospho-tungstic acid, the precipitate treated with milk of lime, and the resulting lime salt filtered off. The residue was evaporated and extracted with strong alcohol, from which free betaïne crystallised out.

Schulze and **Frankfurt** (*Ber.*, xxvi. 2151–2155) have recently described the following process for the isolation of betaïne and choline existing in malt-culms and wheat-germs. The material is extracted with water, lead acetate added so long as a precipitate is produced, the solution acidified with sulphuric acid, and filtered. Phospho-tungstic acid is then added, and the resulting precipitate washed and treated in the cold with milk of lime. The filtrate from the insoluble calcium compounds is treated with carbonic anhydride to remove the excess of lime, filtered, neutralised with hydrochloric acid, evaporated to a syrup, and the latter extracted with hot 90–95 per cent. alcohol. Alcoholic mercuric chloride solution is added to the extract, which is then allowed to stand for several days, when the separated mercury double salt is removed and crystallised from water. The difficultly soluble portion of the salt contains the betaïne, while the easily soluble part consists of the choline compound. These can be separated by repeated fractional crystallisation from water, or by decomposing with sulphuretted hydrogen and treating the hydrochlorides so obtained with cold absolute alcohol. The choline salt dissolves while the betaïne compound remains behind.

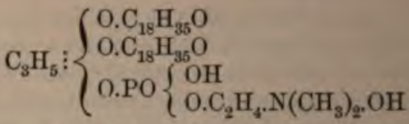
Three kilos. of wheat embryos yielded 5 to 6 grms. of betaïne hydrochloride, the yield of the choline salt being considerably less. Malt rootlets yielded a somewhat less amount of the betaïne salt, but a rather larger quantity of the choline compound.

E. **Jahns** (*Ber.*, xxvi. 1493; *Pharm. Jour.*, [3], xxiv. 245) solated choline and betaïne from worm-seed (*Artemisia Gallica*, Wild.) by the following process:—The seed was extracted with ot water, and the liquor precipitated with lead acetate and soda. rom the filtrate the excess of lead was thrown down by sodium hosphate, the filtrate evaporated to a small bulk, acidulated with lphuric acid and shaken with chloroform, which removed a bitter sinous substance and a little santonin. From the aqueous layer, ixed with a large proportion of sulphuric acid, the bases were ecipitated by potassio-bismuth iodide, the precipitate washed th dilute sulphuric acid, and the choline and betaïne liberated digesting the precipitate with freshly precipitated silver carbone and water. The bases were separated by treating their lorides with absolute alcohol, in which, at the ordinary mperature, the choline salt dissolves freely, while that of betaïne almost insoluble.

LECITHINS OR LECITHINES.

Vauquelin was the first to observe that the brain contains a osphorised fat, which was later obtained from yolk of egg and iare in a crystallised state by **Hoppe-Seyler**, who showed that the same substance, or others closely related to it, occurred very frequently in growing cells, both in the animal and the vegetable kingdom. Thus he isolated lecithins from yeast and various fungi, from seeds, and found it in all the organs and fluids of the human body except the gastric juice, the pancreatic secretion, the urine, and the saliva.[1]

When a lecithin is boiled with baryta-water it is saponified with formation of **choline**, **glycerol-phosphoric acid**, and one or more **fatty acids**, which may be stearic, palmitic, or oleic acids. **Lippmann** obtained **betaïne** instead of choline by the saponification of beetroot-lecithin. Hence it appears that the lecithins are a group of closely-allied compounds, related to each other in much the same manner as the ordinary fats and cholesterins. The constitution of **distearyl-lecithin**, isolated by **Diakonow** from the yolk of egg, is shown by the following formula :—



$$C_3H_5 \vdots \begin{cases} O.C_{18}H_{35}O \\ O.C_{18}H_{35}O \\ O.PO \begin{cases} OH \\ O.C_2H_4.N(CH_3)_2.OH \end{cases} \end{cases}$$

For the preparation of lecithin, Diakonow directs that yolk of egg should be shaken up with ether as long as colouring matter is removed, when the residue is treated with water, filtered, and rapidly washed, and then digested with alcohol at 50°–60° C. The filtered liquid is quickly evaporated to the consistence of a syrup, the residue dissolved in a small quantity of absolute alcohol, and the filtered solution cooled by a freezing mixture. Stearin-lecithin separates gradually in nodular masses or (occasionally) crystalline tablets, while oleïn-lecithin remains in solution. Strecker prepares lecithin by extracting yolk of egg with ether-alcohol, distilling off the ether and adding alcohol to the residue as long as fats and other matters are precipitated. The filtered liquid is treated with an alcoholic solution of platinic chloride containing free hydrochloric acid, which produces a yellow flocculent precipitate of **lecithin platinichloride**, a compound which is soluble in ether, chloroform, or benzene, but insoluble in alcohol. This is purified by repeated solution in ether and precipitation with alcohol, and is then decomposed in ethereal solution by sulphuretted hydrogen. On evaporation to dryness, **lecithin hydrochloride** is obtained as a waxy mass, which is taken up ether-alcohol and shaken with oxide of silver. The resultant er chloride is filtered off and dissolved silver separated from filtrate by sulphuretted hydrogen. On evaporation, pure ithin remains. Lippmann prepared lecithin from beetroot a similar method.

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[1] **C. Schaerges** (*Pharm. Zeit.*, xl. 314) states that the thyroid gland contains a considerable proportion of lecithin, the presence of which he regards as having an important relation to the physiological action of the gland.

Lecithin is a translucent, wax-like, imperfectly crystalline sub- nce, which is very hygroscopic and swells up on treatment h water to form an opalescent liquid or emulsion,[1] which is cipitated or coagulated by various neutral salts.

Lecithin combines both with bases and acids,[2] but its compounds dily undergo decomposition, as also does lecithin itself. An oholic solution of free lecithin decomposes slowly in the cold, l more rapidly on heating, and from a similar solution of lecithin drochloride free fatty acids separate after a time. From an ereal solution of lecithin chloroplatinate, choline chloro- atinate gradually separates on standing. If an ethereal ution of lecithin be shaken with dilute sulphuric acid, choline sses into the acid liquid, while the ether contains distearyl- ycerol-phosphoric acid, $(C_{18}H_{35}O)_2:C_3H_5.PO_4H_2$, a body ich forms a crystalline potassium salt. A similar decomposi- n occurs in the first stage of putrefaction of animal substances taining lecithin (page 324).

By boiling with baryta-water, the molecule of lecithin is split differently, the first products being the barium salts of fatty ds, and the choline ester of glycerol-phosphoric acid, but the ter compound readily undergoes further decomposition with mation of choline and barium glycerol-phosphate.

Lecithin occurs widely distributed in the vegetable kingdom, l is found in the ethereal extracts of plants together with cerides, wax-like products, cholesterin, &c.

For the isolation of lecithin from such extracts, Schulze and kiernik (*Ber.*, xxiv. 71) operate as follows:—Finely-pow-

When lecithin is treated with a moderate quantity of water, the products ibit under the microscope curious filaments, spherules, and other forms, ely resembling the so-called myeline forms observed by Virchow n nerve-fibres are exposed for a long time to the action of water.

imilar myeline forms are produced by "protagon," a highly complex y extracted from ox-brains by means of alcohol. Protagon is stated to e a composition corresponding to the formula $C_{160}H_{308}N_5PO_{35}$, is crystal- ble, and resembles lecithin in yielding choline, fatty acids, and glycerol- sphoric acid as decomposition-products.

As lecithin forms definite (though unstable) compounds with acids, it the characters of a base, and would be more appropriately spelt ithine. In practice, this is very rarely done.

dered plant-seeds (vetch and lupin) are first extracted with ether, when only a portion of the lecithin goes into solution. The insoluble residue is then digested with proof-spirit (in some cases a little alkali being added to neutralise the free acid contained in the seeds), whereby the bulk of the lecithin is dissolved in a fairly pure state.[1] In order to purify the product thus obtained, the solvent is distilled off at 40°–50° C., and the residue treated with cold ether. The lecithin dissolves, and by shaking the solution thus obtained with water the impurities are taken up by the latter. An emulsion, however, forms on shaking the mixture, and crystals of common salt must be added to clear the ethereal solution. This clear solution, when gently evaporated, leaves a residue of lecithin, which is further purified by dissolving it in absolute alcohol and again concentrating. The lecithin separates as a pale yellow product, possessing the characteristic properties of this body; but it could not be obtained in a crystalline form. When saponified by baryta-water, choline, glycerol-phosphoric acid, and fatty acids result, and were separated and identified.[2] Both solid fatty acids and oleic acid were found in the products of saponification, so that the lecithin from plants, like that got from the yolk of eggs, appears to be a mixture of several lecithins.[3]

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[1] B. von Bitto (*Zeits. Physiol. Chem.*) has pointed out the great difficulty attending the complete extraction of lecithin by Schulze's process. After exhausting the substance with ether, he recommends that the residue should be boiled from 20 to 30 times with alcohol.

[2] GLYCEROL-PHOSPHORIC ACID $(OH)_2:C_3H_5.H_2PO_4$, may be obtained synthetically by the action of phosphoric anhydride or glacial phosphoric acid on glycerol. It is produced by boiling lecithin with baryta or caustic soda; and occurs normally in urine (0·015 gramme per litre) and in animal tissues, &c., containing lecithin.

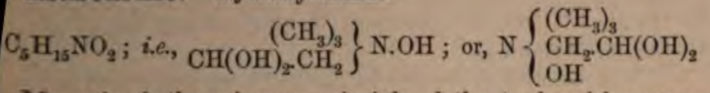
Glycerol-phosphoric acid has not been obtained pure, as it is decomposed on evaporation. In its most concentrated condition it forms a yellowish syrupy liquid of a sweet-acid taste. Glycerol-phosphoric acid is dibasic, and forms salts which are mostly soluble in water but insoluble in alcohol. It may be precipitated as the lead salt.

Calcium glycerol-phosphate, $CaC_3H_7PO_6$, forms a white crystalline powder, freely soluble in cold water but precipitated from its solution on boiling. It has been proposed as a readily assimilable form of phosphorus for medicinal use.

[3] W. Maxwell (*Amer. Chem. Jour.*, xiii. No. 6) has observed that the inorganic phosphorus present in mature seeds becomes reorganised under the action of the processes occurring during incipient growth, and appears in the young plantlet in the organic form as a constituent of lecithin. Maxwell has further observed that the lecithin present in the egg of a hen becomes reorganised under the action of the process of incubation, and is found in the form of a mineral phosphate in the bone of the chicken.

he presence of lecithin in the seeds of plants leads to an error he determination of the fat contained by extraction with ether, he lecithin also goes into solution, though to a varying extent. e error is small in the case of seeds rich in fatty matter, but en the ether extract only amounts to about 2 per cent. and the r cent. of lecithin reaches 1·2 to 1·3, as in the seeds of the tch and of the pea, the error is very considerable, and a determination of the phosphorus in the residue should be made and the uantity of extract free from lecithin thus obtained. One part of $g_2P_2O_7$ represents 7·27 parts of lecithin.

Muscarine. Hydroxy-choline.



$$C_5H_{15}NO_2;\ i.e.,\ \left.\begin{matrix}(CH_3)_3\\ CH(OH)_2.CH_2\end{matrix}\right\}N.OH;\ \text{or},\ N\left\{\begin{matrix}(CH_3)_3\\ CH_2.CH(OH)_2\\ OH\end{matrix}\right.$$

Muscarine is the poisonous principle of the toadstool known as the fly-blown agaric (*Agaricus muscarius*), in which it occurs together with choline. Muscarine is also present in the fungus *Amanita pantherina*, and is a characteristic product, together with ethylene-diamine and gadinine, of the putrefaction of fish. It results from the oxidation of choline by strong nitric acid.

Muscarine forms thin laminæ or irregular crystals. It is very deliquescent, and is soluble in water and alcohol in all proportions, but is insoluble in ether and only with difficulty soluble in chloroform.

The aqueous solution of muscarine is strongly alkaline, absorbs carbon dioxide from the air, and precipitates solutions of ferric and cupric salts.

The salts of muscarine are mostly very deliquescent, and neutral to litmus, except the carbonate, which is strongly alkaline. The salt produced by treating muscarine with hydrochloric acid is formed by the replacement of the hydroxyl-group, OH, by Cl, with elimination of water (compare page 238). The platinum salt contains $(C_5H_{14}NOCl)_2PtCl_4 + 2$ aqua, and forms well-defined octahedra, soluble in alcohol and difficultly soluble in water.

Muscarine salts yield amorphous precipitates with Mayer's reagent, the potassio-iodide of bismuth, with auric chloride, and with phospho-molybdic and phospho-tungstic acids. With Mayer's reagent muscarine salts yield a precipitate which is at first amorphous, but gradually becomes crystalline. Muscarine is not precipitated by tannin, picric acid, or iodised potassium iodide, and is not affected by boiling with dilute acids or alkalies.

According to G. Nothnagel (*Ber.*, xxvi. 801) the artificial muscarine obtained from choline by oxidation with nitric acid agrees with the natural base in crystalline form, solubility, the composition of the platinum and gold salts, and to a large extent in its physiological action; but while artificial muscarine induces paralysis of the intermuscular nerve-terminations in the frog, and myosis in the pupil of the eye of birds, the natural base does not act in either of these manners.

Muscarine is tasteless but very poisonous, the action being narcotic and antagonistic to atropine. The heart of a frog was arrested by 0·00003 gramme of muscarine, but recommenced its action on application of atropine. In its poisonous action muscarine resembles neurine. It produces a flow of saliva and tears, and paralyses and arrests the heart in diastole. Contraction of the pupil, diarrhœa, and emission of urine and semen are other notable symptoms.

Various other fungi besides the fly-blown agaric produce marked symptoms of poisoning, but the active principles do not appear to be of alkaloidal nature; except, perhaps, in the case of *Agaricus ruber*, which is stated by T. L. Phipson to contain a colouring matter (ruberine) and the alkaloid *agarythrine* (*Chem. News*, xlvi. 199).[1]

In man, from 3 to 5 milligrammes of muscarine injected hypodermically produce, in a few minutes, profuse salivation, rapid pulse, nausea, confusion of thought, giddiness, and myosis, but no vomiting or diarrhœa. Applied in small quantity to the eye, muscarine produces derangement of the accommodation but no change in the size of the pupils. Larger quantities cause myosis.

ISOMUSCARINE is an isomer of muscarine obtained by synthetical means (see page 233).

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[1] The autumn fungus, *Agaricus phalloides*, which has not unfrequently been eaten in mistake for mushrooms, with fatal results, is said to owe its poisonous properties to a toxalbumin called phallin (see A. Wynter Blyth, *Poisons: their Effects and Detection*).

[2] For the detection of muscarine in cases of poisoning by the actual base, or by the fly-blown agaric, A. Wynter Blyth suggests that the matters should be treated with water acidulated with hydrochloric acid, and the liquid concentrated to a syrup *in vacuo*. The syrup should then be treated with water, and mercuric chloride added. The excess of mercury is removed from the filtered liquid by sulphuretted hydrogen, and the filtrate evaporated to a syrup, which is then repeatedly extracted with alcohol, and the solution treated with platinic chloride. The filtrate is freed from alcohol, treated with sulphuretted hydrogen, and again filtered, the filtrate concentrated to a small volume, and platinic chloride again added, when the platinum salt of muscarine may be thrown down at once, or on further concentration. Isolation of the muscarine by precipitation with Mayer's solution would, in the opinion of the author, be preferable to the foregoing scheme.

UREA AND ITS ANALOGUES.

Urea is itself of pre-eminent interest and importance as the chief form in which the nitrogen of the food ingested by man and other of the mammalia is eliminated from the system. Urea is also the type of an extensive series of allied bodies and the nucleus of other compounds of natural origin and artificial synthetic formation. The following is a list of the simpler and more typical members of the group:—

CARBAMIC ACID.[1] $\mathrm{CO}\left\{\begin{matrix}\mathrm{OH}\\ \mathrm{NH_2}\end{matrix}\right.$	CARBAMIDE. (Urea.) $\mathrm{CO}\left\{\begin{matrix}\mathrm{NH_2}\\ \mathrm{NH_2}\end{matrix}\right.$	IMIDO-UREA. (Guanidine.) $\mathrm{C(NH)}\left\{\begin{matrix}\mathrm{NH_2}\\ \mathrm{NH_2}\end{matrix}\right.$
ETHYL CARBAMATE. (Urethane.) $\mathrm{CO}\left\{\begin{matrix}\mathrm{O(C_2H_5)}\\ \mathrm{NH_2}\end{matrix}\right.$	ETHYL UREA. $\mathrm{CO}\left\{\begin{matrix}\mathrm{NH(C_2H_5)}\\ \mathrm{NH_2}\end{matrix}\right.$	THIO-UREA. $\mathrm{CS}\left\{\begin{matrix}\mathrm{NH_2}\\ \mathrm{NH_2}\end{matrix}\right.$
PHENYL-URETHANE. (Euphorin.) $\mathrm{CO}\left\{\begin{matrix}\mathrm{O.(C_2H_5)}\\ \mathrm{NH(C_6H_5)}\end{matrix}\right.$	DIALLYL UREA. (Sinapoline.) $\mathrm{CO}\left\{\begin{matrix}\mathrm{NH(C_3H_5)}\\ \mathrm{NH(C_3H_5)}\end{matrix}\right.$	ALLYL-THIOUREA. (Thiosinamine.) $\mathrm{CS}\left\{\begin{matrix}\mathrm{NH(C_3H_5)}\\ \mathrm{NH_2}\end{matrix}\right.$
BIURET. $\mathrm{CO}\left\{\begin{matrix}\mathrm{NH(CO.NH_2)}\\ \mathrm{NH_2}\end{matrix}\right.$	ACETYL-UREA. $\mathrm{CO}\left\{\begin{matrix}\mathrm{NH(CO.CH_3)}\\ \mathrm{NH_2}\end{matrix}\right.$	PHENATOL-UREA. (Dulcine. Sucrol.) $\mathrm{CO}\left\{\begin{matrix}\mathrm{NH.C_6H_4(O.C_2H_5)}\\ \mathrm{NH_2}\end{matrix}\right.$
ALLOPHANIC ACID. $\mathrm{CO}\left\{\begin{matrix}\mathrm{NH(COOH)}\\ \mathrm{NH_2}\end{matrix}\right.$	OXALURIC ACID. $\mathrm{CO}\left\{\begin{matrix}\mathrm{NH.CO(COOH)}\\ \mathrm{NH_2}\end{matrix}\right.$	ALLOXANIC ACID. $\mathrm{CO}\left\{\begin{matrix}\mathrm{NH.CO.CO(COOH)}\\ \mathrm{NH_2}\end{matrix}\right.$
HYDANTOIN. $\mathrm{CO}\left\{\begin{matrix}\mathrm{NH.CO}\\ \mathrm{NH.CH_2}\end{matrix}\right\}$	PARABANIC ACID. $\mathrm{CO}\left\{\begin{matrix}\mathrm{NH.CO}\\ \mathrm{NH.CO}\end{matrix}\right\}$	ALLOXAN. $\mathrm{CO}\left\{\begin{matrix}\mathrm{NH.CO}\\ \mathrm{NH.CO}\end{matrix}\right\}\mathrm{O}$

Guanidine is described on page 282, and biuret on page 250. Thiosinamine is described under mustard oil (page 108), while xaluric acid, parabanic acid, and alloxan are referred to under ric acid. Urea and dulcine are the only remaining members of he group which require further consideration.

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[1] CARBAMIC ACID, $\mathrm{NH_2.COOH}$, is not known in the free state. The mmonium salt is formed by the direct union of carbon dioxide and dry mmonia gases, a second molecule of ammonia uniting with the nascent acid o form *ammonium carbamate*, $\mathrm{NH_2.COO(NH_4)}$. This salt exists in commercial mmonium carbonate, and can be obtained by digesting that compound in trong ammonia for 30 to 40 hours (Divers). Ammonium carbamate is xtremely soluble in water, with which it gradually reacts to form ammoium carbonate, $\mathrm{(NH_4).O.CO.O(NH_4)}$. When heated to about 60° C., t the ordinary pressure, ammonium carbamate is decomposed into ammonia nd carbon dioxide, but when heated under pressure to 130° to 140° it yields rea, as it does also when submitted in aqueous solution to a rapidly alternating current of electricity.

Urea. Carbamide.

$$CH_4N_2O;\ \textit{i.e.},\ CO\left\{\begin{matrix}NH_2\\NH_2\end{matrix}\right.$$

Urea exists ready-formed in the urine of mammals, a
blood, milk, and other animal fluids. It was first ob
synthetically by Wöhler in 1828, being the first of the n
organic bodies prepared by a synthetic process.

Urea may be prepared by a variety of methods, of whi
following are the most important and interesting:—

1. Fresh urine is concentrated at 100° C. to one-tenth
volume, and the insoluble deposit of phosphates and
separated by filtration through cloth. The filtrate is mixed w
equal measure of a hot concentrated solution of oxalic aci
the whole vigorously agitated and allowed to cool. A copious,
coloured precipitate of oxalate of urea is obtained, which is
ated by a cloth filter, slightly washed with cold water and p
The product is dissolved in boiling water, and powdered chalk
till the liquid becomes neutral and effervescence ceases. The
is filtered from the calcium oxalate, warmed with animal ch
filtered, and concentrated by evaporation, avoiding actual h
The urea which deposits on cooling is purified by recrystalli

According to H. J. H. Fenton (*Chem. News*, liii. 13), on treatme
sodium hypochlorite and caustic soda, one-half of the nitrogen of am
carbamate is gradually evolved as gas. The solution then contains
carbamate; and if sodium hypobromite or bromide be next added, the
ing nitrogen is evolved as gas. Fenton suggests this reaction as a
test for a bromide. (Compare page 276.)

Calcium carbamate is precipitated on adding lime and alcohol to a
of ammonium carbamate cooled to 0° C. It forms a crystalline powder,
in water. The solution rapidly decomposes with separation of ca
carbonate.

Salts of carbamic acid occur in serum, and are also stated to be for
the oxidation of leucine, tyrosine, glycocine, and albumin by potass
manganate in alkaline solution.

Ethyl carbamate or *Urethane*, $NH_2.CO.O(C_2H_5)$, results from the a
aqueous ammonia on ethyl carbonate. It is also formed by the a
alcohol at 100° C. on urea or urea nitrate, and may be obtained b
reactions. Traces of urethane exist in urine. Ethyl carbamate m
about 50°, and distils at 182°. It is sparingly soluble in water, but
soluble in alcohol and in ether. Treated in the cold with alcoholic
it yields crystals of potassium cyanate, KNCO. When heat
ammonia, urethane is converted into urea.

Phenyl-urethane, $NH(C_6H_5).CO.O(C_2H_5)$, has been employed medi
as an antipyretic and antirheumatic, under the name of "euph
(Part ii. page 72.) Acetyl and propionyl derivatives of oxyphenyl-ur
have been prepared and proposed as remedies by E. Merck.

2. Liebig and Wöhler's classical method of preparing urea affords an interesting example of rearrangement of the atoms in the molecule. Both ammonium cyanate and urea have an elementary composition corresponding to the empirical formula :—CH_4N_2O. On evaporating an aqueous solution of ammonium cyanate at the temperature of boiling water, the salt suffers molecular change into urea, according to the equation :—$CN.O(NH_4) = CO(NH_2)_2$. The conversion is never quite complete. The reverse reaction occurs to a limited extent when an aqueous solution of urea is boiled, and more completely if silver nitrate be added (compare page 251).

In carrying out Liebig's reaction in practice, it is not necessary to operate on pure ammonium cyanate. Potassium cyanate in strong aqueous solution is treated with an equal weight of ammonium sulphate, and the whole evaporated to dryness on the water-bath. The product is boiled with strong alcohol, which dissolves the urea, leaving a residue of potassium and ammonium sulphates. On concentrating and cooling the alcoholic solution, crystals of urea are deposited. Instead of employing potassium cyanate previously prepared, it may be extemporised by heating a mixture of 28 parts of dehydrated potassium ferrocyanide and 14 parts of manganese dioxide in an iron vessel till it becomes sticky. The product is extracted with cold water, evaporated to dryness with 20·5 parts of ammonium sulphate, and the residue extracted with alcohol as before.

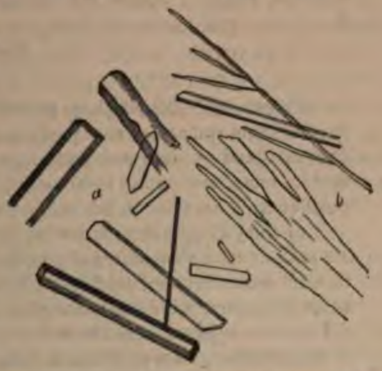

Fig. 7.—UREA. *a*, quadrilateral prisms; *b*, indefinite crystals, as deposited from alcoholic solutions.

J. Williams (*Jour. Chem. Soc.*, xxi. 63) has proposed to employ lead cyanate in place of the potassium salt.[1] It is digested with water and an equivalent quantity of ammonium sulphate at a gentle heat, the liquid filtered from the insoluble lead sulphate, and the filtrate evaporated to the crystallising point.

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[1] The lead cyanate is prepared by fusing the best commercial cyanide of potassium at a very low red heat in a shallow iron vessel, and gradually adding red lead, in small quantities at a time, with constant stirring, so as to avoid much rise of temperature. The product is poured out, finely powdered, exhausted with successive portions of cold water, the solution filtered, and barium nitrate added. The liquid is filtered from the precipitate of barium carbonate, and treated with lead nitrate. The precipitated lead cyanate is washed thoroughly and dried at a gentle heat.

3. Urea has been obtained by passing a current of air mixed with ammonia and benzene vapour over heated platinum wire (*Jour. Chem. Soc.*, xxxix. 471), and by passing ammonia and carbon dioxide through a red-hot tube.

Urea forms transparent, colourless, four-sided, somewhat hygroscopic anhydrous prisms (fig. 7). It is odourless and possesses a cooling saline taste, like that of nitre. When heated to 132° C. urea melts, and at 150° to 160° decomposes with evolution of ammonia and formation of biuret, $C_2H_5N_3O_2$,[1] which on further heating splits into ammonia and ammonium cyanate, leaving a residue containing melanuric acid, $C_3N_3(OH)_2NH_2$, and cyanuric acid, $C_3H_3N_3O_3$, which bears a much stronger

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[1] Biuret, $NH:(CO.NH_2)_2$, is formed when urea is heated to 150°-160°, until the fused substance becomes pasty and ceases to evolve ammonia. On treating the product with hot water, cyanuric acid remains undissolved, and biuret crystallises out on (concentrating and) cooling the filtrate. It may be purified by re-solution in hot water and precipitation with dilute ammonia.

Biuret crystallises from water in long, white, acicular crystals, containing 1 aqua; or from alcohol in anhydrous laminæ. It is sparingly soluble (1:65) in cold water, but very readily (45:100) in boiling water, and is easily soluble in alcohol. Biuret is dissolved unchanged by cold concentrated sulphuric acid.

Biuret in aqueous solution is not precipitated by tannin, nor by solutions of lead or silver. Its most characteristic reaction is the production of a red or violet solution (the tint varying with the relative proportions of biuret and the reagent employed), on adding caustic soda and a few drops of a solution of cupric sulphate or Fehling's solution. This test, often referred to as "the biuret reaction," affords a valuable means of detecting urea (compare page 254).

Biuret is a weak base, forming salts readily decomposed by water. The cyanurate, $B,C_3H_3N_3O_3$, is deposited in needles during the preparation of biuret. It differs from urea cyanurate, for which it has been mistaken, by giving $3NH_3$ instead of $2NH_3$ when boiled with baryta-water, and in evolving 14·8 per cent. of nitrogen instead of 11·5 per cent. when treated with alkaline hypobromite.

When heated to a temperature above 170°, biuret is decomposed into ammonia and cyanuric acid. Heated in a current of hydrochloric acid gas, it yields cyanuric acid, urea, and guanidine, together with ammonia and carbon dioxide:—

$$\underset{\text{Biuret.}}{NH:(CO.NH_2)_2} = CO_2 + \underset{\text{Guanidine.}}{NH:C:(NH_2)_2}$$

According to Fenton, on treatment with alkaline hypobromite, biuret gives off two-thirds of the total nitrogen in the gaseous state, but with hypochlorite only one-third of the nitrogen is stated to be evolved.

ithout change.[1] In a vacuum, urea distils unchanged at

a is soluble in an equal weight of cold water, and in a much antity at 100°. It is also readily soluble in alcohol, and es in amylic alcohol, but it is nearly insoluble in ether, and o in chloroform and volatile oils.

the ordinary temperature, an aqueous solution of pure urea actically no tendency to change, but on boiling, a certain on to ammonium cyanate takes place. The transformation in about an hour, when the decomposition is between 4 and cent. (Walker and Hambly, *Jour. Chem. Soc.*, lxvii. 749). heated with water under pressure, urea undergoes hydro- ith formation of ammonium carbonate:—$CH_4N_2O + 2H_2O =$ $_2CO_3$. In the urine, where the urea is associated with ible organic matter, it readily undergoes a similar change, is the cause of the alkaline reaction of putrid urine. The iacal fermentation of urine has been found to be due to tion of an organised ferment (*Torula ureæ*) in the urine. hange is set up by contact with the stomachs of men, dogs, bits, and has been often occasioned in the bladder by the iction of a septic catheter.

a also yields ammonia when fused with caustic alkali or with soda-lime, a carbonate being formed at the same When heated with a strong mineral acid, urea similarly an ammoniacal salt, carbon dioxide being evolved.

e concentrated nitric acid combines with urea without posing it, but if the acid contain nitrous acid the urea is

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echsel (*Jour. prakt. Chem.* [2], ix. 284) gives the following for- n illustration of the action of heat upon urea:—

a. $2CO(NH_2)_2 = NH_3 + \underset{\text{Biuret.}}{(CO.NH_2)_2:NH}$

b. $CO(NH_2)_2 = NH_3 + \underset{\text{Cyanic acid.}}{CN.OH} + \underset{\text{Ammonium Cyanate.}}{CN.O.(NH_4)}$

c. $CO(NH_2)_2 = H_2O + \underset{\text{Cyanamide.}}{CN.NH_2}$

d. $CN.OH + H_2O = CO_2 + NH_3$

e. $CO_2 + 2NH_3 = \underset{\text{Ammonium carbamate.}}{CO(NH_2).COO(NH_4)}$

f. $2CN.OH. + CN.NH_2 = \underset{\text{Malanuric acid.}}{C_3N_3(OH)_2NH_2}$

g. $3CN.OH = \underset{\text{Cyanuric acid.}}{C_3N_3(OH)_3}$

h. $\underset{\text{Biuret.}}{3C_2H_5N_3O_2} = 3NH_3 + \underset{\text{Cyanuric acid.}}{2C_3N_3(OH)_3}$

resolved into water, nitrogen, and carbon dioxide, according to the following equation:—$CH_4N_2O + N_2O_3 = 2H_2O + 2N_2 + CO_2$. With Millon's reagent the reaction occurs promptly and completely, and may be employed for the determination of urea.

Chlorine, bromine, hypochlorites, and hypobromites decompose solutions of urea with evolution of nitrogen. One of the best practical methods of determining urea in urine is based on this reaction (page 263).

A compound of urea with sodium chloride, of the formula $CH_4N_2O,NaCl,H_2O$, separates in brilliant rhombic crystals when mixed solutions of urea and common salt are evaporated. This compound sometimes crystallises from concentrated human urine.

SALTS OF UREA.

Urea is a somewhat feeble base. It forms a well-defined series of salts, all of which are more or less soluble. Many of them are decomposed by excess of water, and the aqueous solutions are in all cases acid to litmus. The *nitrate* and *oxalate* of urea crystallise well, and are employed for the isolation and detection of urea. Urea also combines with metallic salts, the compounds being mostly soluble, with the exception of those with mercuric nitrate.

Urea Nitrate, CH_4N_2O,HNO_3, separates in crystals when moderately strong nitric acid is added to a concentrated aqueous solution of urea, and the liquid cooled. The compound forms brilliant white scales or plates, or, if the deposition is slow, prismatic crystals. When nitric acid and urea are brought together on a microscope-slide, and the reaction observed under a low power, the formation of obtuse rhombic octahedra is first noticed, the angles being *constantly* 82°. These octahedra change to rhombic and hexagonal tables, either separate or superposed (see fig. 8, *a*), but also having angles of 82°. For the formation of nitrate of urea from normal urine, it is sufficient to concentrate the liquid to about one-fourth of its volume, filter after cooling from the precipitated urates, &c., and add nitric acid to the cold filtrate. Nitrate of urea is unalterable in the air. It is readily soluble in water, forming a solution of acid reaction and taste. It is also soluble in alcohol, but only very slightly soluble in presence of nitric acid. Oxalic acid precipitates urea oxalate from concentrated solutions of the nitrate.

Urea Oxalate, $(CH_4N_2O)_2,C_2H_2O_4$, is readily formed on mixing concentrated solutions of urea and oxalic acid. From urine it may be prepared by adding oxalic acid to the concentrated and filtered liquid. Urea oxalate forms thin crystalline plates (see fig. 8, *b*), usually grouped together, but sometimes in well-formed separate

s microscopic appearance is not unlike that of the
ea, but the forms are less characteristic, and the angles
. Oxalate of urea is soluble with difficulty in cold
issolves readily at a boiling heat. It is less soluble in
f oxalic acid than in pure water. The salt dissolves
of alcohol, but is quite insoluble in amylic alcohol.
solution of urea in amylic alcohol (such as will result
ating urine to dryness, heating the residue with amylic
filtering) be treated with a cold saturated solution of
in amylic alcohol, urea oxalate will be precipitated in
ls (see further, page 256).

a very deliquescent crystalline mass, formed by the
ydrochloric acid gas on urea. It is decomposed by
its constituents. $B,HAuCl_4,+1$ aqua forms orange-

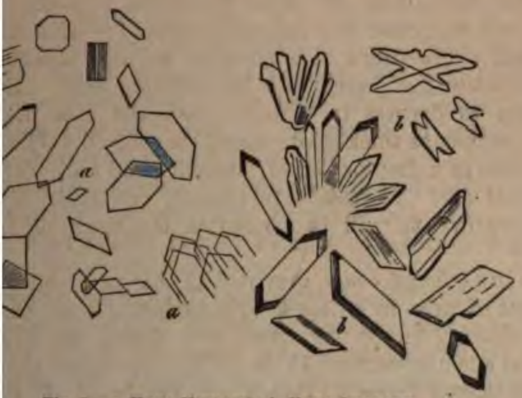

Fig. 8.—*a*, Urea Nitrate; *b*, Urea Oxalate.

or needles, very soluble in water, alcohol, and ether.
2aq forms yellow needles, very soluble in hot water.
obtained in large, very soluble, rhombic crystals on
pig's urine or mixed solutions of urea and phosphoric

s not appear to form any definite compound with uric
cording to Klemperer (*Pharm. Zeit.*, xli. 30) urea is
ous than piperazine, or lysidine (pages 198, 200), as
cal diuretic and solvent of uric acid.

N OF UREA.

duces no precipitate with tannin or other general
the alkaloids. It gives no reaction with either neutral
ad acetate, and does not reduce Fehling's solution

even on boiling. It gives no colour-reactions with oxidising agents.

If a fragment of solid urea be moistened with a concentrated solution of furfurol, and a drop of strong hydrochloric acid (sp. gr. 1·10) be then added, a fine violet coloration is produced (Schiff, *Berichte*, x. 774).

If a residue containing urea be heated for some time to a temperature not exceeding 160° C., the product will contain biuret. On dissolving it in water, adding caustic soda, and then dropping in a dilute solution of cupric sulphate, a violet or red coloration will be produced if urea were originally present.

If an aqueous solution of urea be heated with silver nitrate, a white precipitate of silver cyanate is formed, soluble in boiling water, while the filtered liquid is found to contain ammonium nitrate:—$CO:N_2H_4 + AgNO_3 = CN.AgO + NH_4NO_3$.

For the recognition of urea in dilute aqueous solution Bloxam has suggested the following method:—If a nitrate be present, add a few drops of ammonium chloride solution, but if absent, acidulate the liquid with hydrochloric acid. Evaporate the solution to dryness in a watch-glass, and heat the residue cautiously as long as thick white fumes are evolved. Dissolve the cooled residue in a drop or two of ammonia, add a drop of barium chloride, and stir. If urea were present, crystalline streaks of barium cyanurate will be formed in the track of the glass rod.

Musculus (*Compt. rend.*, lxxviii. 132) has proposed to detect the presence of urea in a liquid by introducing a test-paper prepared by filtering fermenting urine and drying the filter-paper employed at a temperature of 35° to 40° C. The paper entraps the special torulaceous ferment (*Torula ureæ*) observed by Pasteur and Van Tieghem, which assumes the form of small spheres and transforms urea into ammonium carbonate. The paper thus prepared can be kept in a dry state for several weeks without losing its activity. To determine urea, slips of the impregnated paper are introduced into the exactly neutralised liquid, and after a lapse of six hours the ammonia generated is determined by titration with standard acid. By impregnating the prepared paper with turmeric, it may be employed for the qualitative detection of urea; for a solution of urea in 1000, or even in 10,000, parts of water causes the paper to become more or less brown after an immersion of a few minutes. Proteids give no ammonia till after a long interval of time, and uric acid, xanthine, hypoxanthine, &c., are unaffected by the ferment.

On mixing a solution of urea with one of neutral mercuric nitrate, a white flocculent precipitate is obtained. This has a composition dependent on the concentration of the liquid, containng, according to the conditions of its formation, 1, 1½, or 2 molecules of mercuric oxide to 1 of urea. If, however, the addiion of the mercuric nitrate be continued as long as precipitation ccurs, and sodium bicarbonate be added in quantity sufficient to neutralise the nitric acid set free, the precipitate has the composiion $CH_4N_2O,2HgO$. The end of the reaction is indicated by the yellow colour developed from the formation of basic nitrate of mercury. Liebig's method of determining urea is based on his reaction (see page 259). The mercuric oxide compounds of rea are decomposed by sulphuretted hydrogen with precipitation f mercuric sulphide and liberation of urea, a fact which may be tilised for the isolation of the base from urine.

Urea is not precipitated by a solution of mercuric chloride. he addition of mercuric nitrate to a soluble chloride results otentially in the formation of mercuric chloride. As sodium hloride is present in urine, mercuric nitrate produces no precipiate of Liebig's compound in that liquid until sufficient has been dded to react fully with the chloride present. On this fact iebig based a method for determining chlorides in urine.

Mercuric acetate gives no precipitate with urea in the cold, and he separation is very incomplete on boiling.

The recognition of urea in animal fluids is usually based on the eparation of the nitrate or oxalate. If the quantity of urea resent is sufficient for the preparation of these salts in such mount as to allow a study of their properties, the determination f urea can be effected. On the other hand, if the quantity of rea present be very minute, as in the case of blood and of all cretions and excretions other than urine, it is not always easy to void error.

For the detection of urea in blood-serum or other serous fluids, he liquid should be mixed with three or four measures of alcohol, hich precipitates the albuminous matters.[1] The filtered liquid evaporated on the water-bath, and the residue exhausted with bsolute alcohol. The alcoholic solution is evaporated on a watch-lass, and if foreign matters show themselves, the treatment with bsolute alcohol is repeated. The extract is evaporated nearly to ryness on a watch-glass, the residue taken up with water, and any hosphates precipitated by addition of baryta-water. Carbon dioxide s passed through the filtered liquid, which is then boiled, again filtered, and evaporated on the water-bath to a syrup. The residue is divided into two or three portions, which are treated respectively with nitric acid and with oxalic acid, and the products examined under the microscope for the recognition of the characteristic crystalline forms of urea nitrate and oxalate, as shown in figs. 8*a* and 8*b* (page 253).

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[1] In some cases it is desirable to effect a preliminary separation of the bulk of the proteids by acidulating the liquid with acetic acid and boiling.

In carrying out the foregoing process, it is very important to study carefully the crystals supposed to be urea nitrate, and, whenever possible, to dissolve and test them with mercuric nitrate. Under certain conditions, and especially in presence of extractive matters, one may meet with nitrates of alkali-metals which resemble in their microscopic appearance the crystals of nitrate of urea. The inorganic salts are distinguished from the latter by their behaviour on ignition, and by the presence of a notable quantity of potassium or sodium in the ash, which will have an alkaline reaction. On the other hand, if a crystal of true nitrate of urea be dissolved in water and treated with a concentrated solution of oxalic acid (which may be effected under the microscope), crystals of oxalate of urea will be gradually formed.

The value of oxalic acid as a reagent for the isolation and recognition of urea is considerably enhanced if advantage be taken of the sparing solubility of urea oxalate in a mixture of alcohol and ether. A still better method is to heat the alcoholic extract to be tested with a small quantity of amyl alcohol, and then treat the solution, decanted or filtered if necessary, with a cold saturated solution of oxalic acid in amyl alcohol. The urea oxalate is precipitated in small crystals, which redissolve on warming the liquid, and on cooling separate out in a condition suitable for microscopic examination. The process may be modified by treating the solution of urea in amylic alcohol with one of oxalic acid in anhydrous ether. Precipitation takes place abundantly and quickly, but the crystals are usually small and imperfect. The oxalic acid may be added in powder, the liquid heated and thoroughly cooled, and the excess of oxalic acid removed from the precipitate by treatment with anhydrous ether. The method is capable of being employed quantitatively. The amylic alcohol used in the process must not develop a red or brown colour with oxalic acid, and should be free from water and ethylic alcohol.

Determination of Urea.

The determination of the urea contained in urine is often of great physiological and pathological interest, since the whole of the nitrogen contained in the effete nitrogenised tissues and of the food digested is ultimately eliminated by the kidneys.

From 85 to 90 per cent. of the total nitrogen contained in normal human urine exists in the form of urea,[1] the remainder being divided between uric acid, hippuric acid, xanthine, creatinine, &c. In the urine of herbivorous mammals the uric acid is replaced by hippuric acid, while the nitrogen of birds and reptiles is eliminated chiefly in the form of uric acid instead of as urea.

As urea is the predominant nitrogenous constituent of normal human urine, it is evident that for many purposes its determination will afford sufficient information as to the amount of nitrogen passing away in the urine.

In view of Schröder's hypothesis that the liver-cells form urea from ammonium carbonate, Mörner and Sjöquist (*Skandinav. Archiv. Physiol.*, ii. 438; abst. *Jour. Chem. Soc.*, 1891, page 758) have determined the relative amounts of urea and ammonia in the urine excreted in various liver diseases. In cases of cirrhosis, syphilis, and cancer of the liver there was an increased amount

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[1] W. Camerer (*Zeit. Biol.*, xxiv. 306; *Jour. Chem. Soc.*, liv. 518) has recorded the amount of total nitrogen contained in normal urine, and has compared it with that eliminated in the form of urea. Thus the mixed urine from a number of persons measured on the average 1840 c.c., had a specific gravity of 1·016, and contained:—

Total nitrogen, . .	16·06 grammes per diem.	= 0·873 per cent.
Nitrogen as urea, . .	14·15 ,,	= 0·769 ,,
Nitrogen in other forms,	1·91 ,,	= 0·104 ,,

The result of a large number of observations by Russell and West (*Proc. Royal Soc.*, xxx. 439) on various cases of disease was to prove that the relation of the ureal to the total nitrogen of urine is approximately constant, except in rare cases of acute yellow atrophy of the liver; and even in these it is doubtful whether the observed replacement of the urea by leucine and tyrosine is a constant phenomenon. In a case of acute fatty atrophy of the liver the urea was still normally formed, while leucine and tyrosine were absent. The following percentages of the total nitrogen existed as urea, according to the observations of Russell and West, which were obtained by the hypobromite process:—

Pneumonia (6 cases), 90 per cent.; jaundice (Case 1), 85·7; jaundice (Case 2), 90·2; albuminuria (2 cases), 86·0; collected cases, 93·8; dieted cases, 90·1; and mean of all, 89·3 per cent. The mean, excluding the jaundice and albuminuria cases, was 91·3 per cent.

Pflüger and Bohland found that 13·4 per cent. of the total nitrogen of urine was in forms other than urea. K. Bohland (*Pflüger's Archiv.*, xliii. 30), by improved methods of determination, obtained from thirteen series of urines, many of them from patients suffering from fevers, the following mean results:—15·54 per cent. of the total nitrogen did not exist as urea; 0·065 was present as pre-formed ammonia; 6·51 per cent. was precipitated by phospho-tungstic acid, and 4·40 was contained in the filtrate.

of ammonia and a lessened amount of urea found; but in cases where no liver disease was present an increase of ammonia in the urine was sometimes noted; for instance, in a case of fatty heart, one of pyopneumothorax, and especially in a case of tetanus. In these researches, Mörner and Sjöquist determined the urea by the method described on page 278.

The *amount* of urea excreted in the urine varies considerably with the diet, being increased by nitrogenous foods. The weight of urea excreted per diem by an adult man on mixed diet ranges from 25 to 40 grammes, the average being about 33 grammes (500 grains). On a diet poor in proteids the excretion of urea may fall to 15 to 20 grammes, while on a flesh diet the daily output may rise to 100 grammes. The proportion of urea in human urine averages about 2 per cent., but dog's urine is stated to contain 10 per cent.

A large excretion of urea, if long continued, points to *increased tissue-metabolism* or to surplus nitrogenous ingesta, but a temporary increase may be simply due to increased urination. Similarly, diminished excretion of urea may be due to diminished metabolism or to retention of urea in the system (as in uræmia).[1]

A great number of methods have been devised for the determination of urea, and of these the following deserve notice:—

1. The precipitation of urea in the form of oxalate is a convenient way of isolating the base from complex mixtures, and under certain conditions gives very fair results. The best method of applying it has already been described (see page 256).

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[1] A great number of observations have been recorded of the influence of drugs, diseases, and other conditions on the proportion of urea excreted. The results have been classified by W. D. Halliburton (*Chemical Physiology and Pathology*, 1891) as follow:—

An increased excretion of urea occurs:—

1. After administration of dilute sulphuric acid, potassium chloride, ammonium salts (especially with food), small doses of phosphorus, arsenic, antimony, morphine, codeine, or large doses of quinine. 2. After poisoning by phosphorus or arsenic. 3. From application of cold to the skin; after hot baths; from increase of oxygen inhaled; from excessive muscular work. 4. In diseases, as at the commencement of acute febrile diseases, up to the acme of the fever; during the paroxysms of intermittent fever (ague); in diabetes.

A decreased excretion of urea occurs:—

1. After administration of small doses of quinine. 2. During the sinking of the fever in acute febrile diseases; in most chronic and debilitating diseases (anæmia, syphilis, phthisis, dropsical affections, &c.); towards the fatal termination of most diseases (5 to 6 grammes daily); in uræmia (when the excretion may entirely cease); in diabetic coma; and in all degenerative changes of the liver, especially in acute yellow atrophy.

2. Urea is converted into ammonia when heated with strong sulphuric acid. The reaction having been effected, the ammonia may be determined by any known method. Thus, it may be distilled off after addition of soda or lime, and the distillate titrated with standard acid; or the resultant ammonia may be converted into chloroplatinate. In accurate experiments on urine the uric acid should be previously separated.

3. *Bunsen's method* of determining urea is capable of yielding very accurate results. Benzoic and sulphuric acids, ammoniacal salts (other than ammonium carbonate), glucose, albumin, and extractive matters do not interfere with the estimation. Carbonates must be got rid of by heating the slightly acidulated urine to boiling, and in accurate experiments interfering bodies should be precipitated by phospho-tungstic acid. The method, as applied by Bunge to urine, is as follows:—30 c.c. measure of urine is treated with 10 c.c. of a cold saturated solution of barium chloride containing some ammonia. The liquid is passed through a dry filter, and 20 c.c. of the filtrate (= 15 c.c. of urine) are introduced into a stout glass tube containing about 3 grammes of solid and chemically pure barium chloride. The tube is then sealed and heated to a temperature of 220° C. (160° is sufficient) during four hours. The barium carbonate formed is transferred to a filter, washed, and dissolved in hydrochloric acid, together with the portion which adheres to the interior of the tube. The solution is diluted, precipitated by dilute sulphuric acid, and the resultant barium sulphate is collected and weighed. Its weight, multiplied by 1·717, gives the grammes of urea in the 100 c.c. of the sample of urine.

4. *Liebig's volumetric method* for the determination of urea in urine is based on the fact that on adding a neutral solution of mercuric nitrate to one of urea a compound is produced containing one molecule of urea to two of mercuric oxide (page 255). This body is white, and nearly insoluble in water. By employing a standard solution of mercuric nitrate, and using sodium bicarbonate to indicate the end of the reaction, the volumetric determination of urea becomes possible. There are, however, various sources of error in the process, which, if not carefully avoided or allowed for, may cause serious deviations from the truth. The following conditions must therefore be complied with. *Phosphates* must be previously removed by treating the urine with a suitable precipitant. *Chlorides* prevent the precipitation of the urea compound, until sufficient mercury solution has been added to convert them into mercuric chloride. An allowance for this purpose must accordingly be made. *Nitric acid* is liberated in the reaction, and must be neutralised during the titration. The manner of effecting this

is not a matter of indifference, the best plan being that of Pflüger, who prescribes the following way of employing Liebig's method of titrating urine for the content of urea :—40 c.c. of the filtered urine, free from albumin, should be treated with 20 c.c. of a mixture of two measures of cold saturated baryta-water with one of a saturated solution of barium nitrate. The liquid is passed through a dry filter to separate the precipitated phosphates, sulphates, carbonates, magnesia, &c., and 15 c.c. of the clear filtrate (=10 c.c. of urine) made just neutral to litmus by cautious addition of nitric acid. If the filtrate be not alkaline, insufficient barium solution has been used, and a fresh experiment should be commenced by mixing 20 c.c. of urine with an equal measure of the barium solution, and taking 20 c.c. of the filtrate.

The *standard solution of mercuric nitrate* is prepared by dissolving 77·2 grammes of mercuric oxide in the smallest possible quantity of nitric acid.[1] The excess of acid should be exactly neutralised by adding caustic soda, using methyl-orange as an indicator, and the solution diluted with water to 1 litre. 1 c.c. of this solution represents 0·010 gramme of urea.

The phosphate-free liquid corresponding to 10 c.c. of the original urine is placed in a beaker, and the standard solution of mercury gradually added. From time to time a drop of the liquid containing the suspended white precipitate is placed on a glass plate so as just to touch a drop of a thick mixture of water with sodium bicarbonate. The glass plate should be placed on a black cloth. At first the urine-mixture will retain its snow-white colour, but a point at length occurs when a yellow colour is produced at the junction of the drops, which disappears on stirring the drops together with a glass rod. Further cautious additions of the mercury solution are made till a permanent faint yellow colour is obtained. A solution of normal sodium carbonate is then run into the beaker in quantity sufficient to saturate the acid set free from the volume of mercury solution employed, the point of neutrality being ascertained by litmus-paper or methyl-orange. Addition of the mercury solution is then continued till a drop of the liquid gives a yellow colour with the indicator. It is important to neutralise the liquid as soon as possible after the addition of the mercury solution; and as this is difficult in a first experiment, the titration should be repeated, the volume of mercury solution previously found necessary being added at once, and

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[1] Or 96·855 grammes of mercuric chloride may be dissolved in water, precipitated by excess of dilute caustic soda, the mercuric oxide washed by decantation till free from chlorides, and dissolved in a slight excess of nitric acid.

ediately followed by an addition of the proper volume of
m carbonate solution. A very small further addition of the
ury solution will then suffice to complete the reaction.
ating in this way, Pflüger (*Zeitschr. anal. Chem.*, xix. 375)
d the process to give very accurate results, but, under certain
itions, corrections become necessary, as follow :—

rrection for concentration.—When more than 20 c.c. of mer-
solution has been found necessary for 10 c.c. of urine, showing
presence of more than 2 per cent. of urea, 1 c.c. of water
ld be added to the ureal solution for every 2 c.c. of mercury
ion employed above 20 c.c. Thus, if 30 c.c. have been used,
15 c.c. of clarified urine should be diluted with 5 c.c. of
r. If less than 20 c.c. of mercury solution be required, for
y 1 c.c. under that volume 0·025 c.c. should be deducted from
neasure of standard solution actually employed before calcu-
g to urea.

rrection for chlorides.—When the proportion of sodium
ide exceeds 1 gramme per 100 c.c. of the urine, a deduction
c.c. should be made from the total quantity of mercury solu-
employed before calculating to the equivalent of urea. A
accurate plan is to determine the chlorides in a separate
on of the urine,[1] and to add to the filtrate from the baryta

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irect precipitation of urine by silver nitrate is not applicable to the
nination of the contained chlorides, since much organic matter is thrown
with the silver chloride. The simplest plan generally available is to
rate 20 c.c. of the urine to dryness in platinum with 3 to 4 grammes of
sium nitrate free from chlorides. On gently heating the residue, the
ic matter is oxidised by the oxygen of the nitre, and on raising the
rature to incipient redness complete combustion of the carbonaceous
r results, and a perfectly white product is obtained. This, when cold,
ated with hot water, the solution acidulated with nitric acid, a little
red chalk added, and the whole thoroughly agitated till neutral to
s. The liquid is then diluted to 100 c.c., and passed through a dry
Fifty c.c. of the filtrate (=10 c.c. of the original urine) should then be
l in a porcelain basin and two drops of a saturated solution of neutral
sium chromate added. A standard solution of silver nitrate containing
grammes of pure $AgNO_3$ per litre is then gradually added, with con-
stirring, until the lemon-yellow colour of the contents of the basin
es to reddish-yellow. This point indicates the conversion of the whole
chlorides present into white silver chloride, AgCl, and the commence-
of the formation of the red silver chromate, Ag_2CrO_4. Every 1 c.c. of
ilver solution used represents 0·010 gramme of sodium chloride in the
. of urine employed (=0·100 gramme per 100 c.c.). Hence if 7·0 c.c.
uired, the urine contains 0·70 per cent., which figure, multiplied by
, equals 3·06 grains of sodium chloride per fluid ounce.
he process has been carried out on the quantities above directed, it will

precipitate previously neutralised by nitric acid just sufficient of a standard solution of silver nitrate to precipitate the chlorides in that volume of urine. The titration with the mercury solution is then conducted without filtering from the silver chloride. Or 15 c.c. of the filtrate from the baryta precipitate may be exactly neutralised by nitric acid, and the mercury solution cautiously added till a faint permanent turbidity is produced. This point corresponds with the completion of the reaction between the sodium chloride and mercuric nitrate, and it is only when this is effected that the compound of mercuric oxide and urea begins to form. A titration of another 15 c.c. of the filtrate is then conducted in the ordinary way, but only the volume of mercury in excess of that previously ascertained to be required for the production of the turbidity is taken into account in calculating the urea.

Albumin, if present in the urine to be treated by the mercury process, must be previously removed by rendering the liquid distinctly acid to litmus (if not already sufficiently acid) by cautious addition of acetic acid (avoiding a large excess), boiling for a few minutes, and filtering the liquid through a dry filter.

Ammonium salts interfere with the determination of urea by the mercury process. Hence, if present in the urine in notable quantity, the filtrate from the baryta precipitate should be evaporated to dryness on the water-bath, and the residue dissolved in a quantity of water equal to that of the liquid before evaporation. The titration is then proceeded with in the usual manner.

The results yielded by the mercury process of determining urea are affected by so many causes that, in the author's opinion, the method cannot be relied on as more than approximately accurate unless steps are taken to remove the interfering bodies, thus complicating the process very materially. Vogel is of opinion that

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be necessary to add the same volume of silver solution to 15 c.c. of the filtrate from the baryta precipitate as was found necessary to produce a reddish-yellow end-reaction in the titration of the chlorides by silver solution.

The chlorides contained in the urine are largely dependent on the quantity of common salt taken with the food, but a portion of them are derived from chlorides of potassium and sodium naturally present in the food. In cases of pneumonia the chlorides almost entirely disappear from the urine, while the sputum contains an excessive amount. Bromides and iodides, which are not natural constituents of urine but appear after administration of medicines containing them, react like chlorides with silver nitrate. Where large quantities of these salts have been taken, the perturbation of the urea determination by the mercury process is so great as to render the method valueless.

r may, in some cases, amount to 20 per cent. of the total esent. Thudichum admits the error, which he attributes resence of the bodies described by him under the names of ne and cryptophanic acid, the latter substance alone being to cause a probable error of from 5 to 10 per cent. *ogy of the Urine).* J. L. W. Thudichum (*Med. Press,* 1, 1895, p. 272) proposes that the urine should be first with "mercuramine" (Millon's base),[1] which is stated to all acids, both organic and inorganic. The filtrate, which all basic bodies in an uncombined state, is then to be de- l with animal charcoal, and the urea determined in the by mercuric nitrate solution. Operating in this manner, ıum asserts that the corrections for chlorides and dilution baryta solution become unnecessary, since all interfering are removed. It is stated that the determination of urea n be effected with "ideal accuracy and ease." Evidently hod is seriously complicated by the preliminary procedure ed by Thudichum, and his statements require careful ion before the process thus modified can be relied on.

ypobromite Process of determining Urea.

foregoing methods of determining urea now receive only ıal application, since the process next to be described is far ıpid and convenient, and gives results sufficiently correct majority of purposes, though, in the opinion of the who has had great experience of it, the accuracy of the in its ordinary form has been exaggerated.

process is based on one devised by Knop and Wolf *Centralb.*, 1860, page 257) for the estimation of ammonia,[2] dependent on the reaction between urea and a strongly solution of sodium hypobromite, whereby sodium bromide,

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employment of Millon's base, mercurammonium hydroxide, for the ion of urine, appears to have been first suggested by Thudichum in To full description of the process appears to have been published in e usual channels, but a short note of it was printed in the *Medical* February 13, 1889. Thudichum there directs the base to be pre- saturating yellow mercuric oxide with ammonia, and washing and he resultant canary-yellow compound in an atmosphere free from ioxide. He ascribes to the product the formula $Hg_4O_3N_2H_4+3H_2O$. tly the reagent is simply shaken with the urine to be clarified.

e chemists employed a solution of sodium hypochlorite to which was added, operating in an apparatus called by them an "azotometer," nd the method inapplicable to urea !

ler, in 1853, described a process for the determination of ammonia by treating the sample with bleaching powder solution and measur- ıitrogen evolved.

water, carbon dioxide, and nitrogen are produced, according to the equation :—$3NaBrO + CH_4N_2O = 3NaBr + 2H_2O + CO_2 + N_2$.

The carbon dioxide gas is absorbed by the excess of caustic alkali employed, so that, under the conditions of the experiment, pure nitrogen gas is evolved.

The hypobromite method of determining urea has been the subject of numerous investigations, with results which are generally favourable, but which present some curious anomalies.

The literature of Knop's process, in its various modifications, is very extensive. The following is a list of papers in English and American periodicals which has been compiled, at the author's request, by A. R. Tankard. Many of the references are to abstracts of foreign papers, which are more accessible than the originals.

Year.	*Author.*	*Reference.*	*Remarks.*
1854	E. W. Davy,	*Phil. Mag.*, [4], vii. 385.	Decomposition of urea by sodium hypochlorite; gas measured over brine.
1871	G. Hüfner,	*Jour. Chem. Soc.*, xxiv. 162.	New apparatus, employing hypobromite, and gentle heat to assist reaction.
1871	J. E. Reynolds,	*Chem. News*, xxxvii. 137.	New apparatus for use with hypobromite.
1873	Yvon,	*J. C. S.*, xxvi. 411.	Ureometer, using hypobromite; gas collected over mercury.
1874	Russell & West,	*J. C. S.*, xxvii. 749.	New form of apparatus, using hypobromite. Yield, 92 per cent.
1875	G. Schleich,	*J. C. S.*, xxviii. 483.	Modification of Hüfner's apparatus.
1875	R. Apjohn,	*Chem. News*, xxxi. 36.	Simple apparatus for medical practitioners.
1875	E. M. de la Source,	*J. C. S.*, xxviii. 916.	Modification of Yvon's ureometer.
1876	J. G. Blackley,	*J. C. S.*, xxx. 466.	Modification of Russell & West's apparatus.
1877	A. Dupré,	*J. C. S.*, xxxi. 534.	Apparatus almost identical with Apjohn's.
1877	Simpson & O'Keeffe,	*J. C. S.*, xxxi. 538.	Apparatus for rough estimations, employing Russell & West's tube.
1877	Yvon,	*J. C. S.*, xxxii. 226.	Calcium hypochlorite is preferable to sodium hypochlorite as a reagent for urea.
1878	H. J. H. Fenton,	*J. C. S.*, xxxiii. 300.	Action of hypochlorites on urea.
1878	J. E. Reynolds,	*Chem. News*, xxxvii. 135.	New forms of apparatus for the hypobromite method.
1878	W. Foster,	*J. C. S.*, xxxiii. 470.	Action of alkaline hypobromite on urea, ammonium salts, and oxamide.
1878	G. Hüfner,	*J. C. S.*, xxxvi. 405.	A formula for correction owing to retention of a portion of the nitrogen in estimations by hypobromite.
1879	H. J. H. Fenton,	*J. C. S.*, xxxv. 12.	Comparison of reactions of hypochlorite and hypobromite with various nitrogen compounds.
1879	W. Foster,	*J. C. S.*, xxxv. 121.	Action of alkaline hypobromite on urea, ammonium salts, and oxamide.
1879	C. Méhu,	*J. C. S.*, xxxvi. 985.	Addition of glucose or cane-sugar causes evolution of total nitrogen.
1879	G. Esbach,	*J. C. S.*, xxxvi. 1067.	Gas evolved by hypobromite and glucose varies with the amount of glucose added.
1880	A. Fauconier,	*J. C. S.*, xxxviii. 513.	Addition of glucose, but not cane-sugar, gives 100 per cent. nitrogen, owing to reduction of nitrate.
1880	Jay,	*J. C. S.*, xxxviii. 513.	Glucose gives off gas with hypobromite.

Author.	*Reference.*	*Remarks.*
Méhu,	*J. C. S.*, xxxviii. 681.	To each part of urea present 10 parts by weight of cane-sugar should be added.
Tattersall,	*Year-Book Pharm.*, 1880, 144.	Too high results are obtained if glucose is present in urine.
Esbach,	*J. C. S.*, xl. 316.	Glucose gives off gas with hypobromite, but cane-sugar does not.
uinquand,	*J. C. S.*, xl. 1085.	Excess of hypobromite is added, then slight excess of alkaline arsenate, and the excess of this reagent determined by hypobromite with indigo sulphate as indicator.
J. Wormley,	*Chem. News*, xlv. 27.	Action of glucose and cane-sugar with hypobromite, and a discussion on Apjohn's form of apparatus.
R. Duggan,	*J. C. S.*, xlii. 778.	If the bromine be added to the soda and urea solutions previously mixed, the yield of nitrogen is increased. Yield, 99 per cent.
Arnold,	*J. C. S.*, xlii. 1141.	Excess of alkali facilitates decomposition of urea. Yield of nitrogen, 91 per cent.
W. Gerrard,	*Pharm. Jour.*, [3], xv. 464.	Simple form of apparatus, called a "ureometer."
R. Squibb,	*Ephemeris*, ii. 448.	Simple form of apparatus, using sodium carbonate and hypochlorite. All nitrogen evolved.
H. Allen,	*Pharm. Jour.*, [3], iv. 179.	Application of nitrometer to estimation of urea by hypobromite.
B. Lyons,	*Analyst*, x. 150.	Adds potassium bromide to sodium hypochlorite.
Lange,	*J. S. Chem. Ind.*, iv. 495.	Use of nitrometer. Yield, 91 per cent.
J. Hamburger,	*J. C. S.*, xlviii. 450.	Adds standard hypobromite, and titrates back with standard sodium arsenite.
Jacobi,	*J. C. S.*, l. 104.	Discussion on use of hypobromite for estimations of urea, &c. Yield, 92 per cent.
flüger & Schenck,	*J. C. S.*, l. 396.	Hamburger's method is of little use.
Salkowski,	*J. C. S.*, l. 396.	Modification of the ordinary hypobromite method.
D. Green,	*J. C. S.*, l. 747.	Error from unabsorbed carbon dioxide.
Knop,	*Jour. Soc. Chem. Ind.*, v. 505.	New apparatus for hypobromite process, called an azotometer.
J. H. Fenton,	*Chem. News*, liii. 13.	Reaction of carbamates with hypochlorites and hypobromites.
J. H. Fenton,	*Chem. News*, liii. 193.	Detection of bromides by mixed carbamate and hypochlorite.
E. Saul,	*Pharm. Jour.*, [3], xvii. 858.	Analysis of urine; new forms of apparatus for hypobromite method.
Frutiger,	*J. S. C. I.*, vi. 149.	New apparatus for hypobromite process.
A. Doremus,	*Chemist and Druggist*, xxx. 729.	Rough apparatus for use of medical practitioners.
flüger & Bohland,	*J. C. S.*, lii. 90.	Urine should be previously treated with phospho-tungstic acid. Pure soda and bromine should be used.
J. H. Fenton,	*Proc. Cambridge Phil. Soc.*, 1888, page 307.	The metameric transformation of ammonium cyanate.
tainer,	*Pharm. Jour.*, [3], xvii. 600.	Frothing in hypobromite process due to albumin.
aul; Davis,	*Pharm. Jour.*, [3], xvii. 620.	Frothing in hypobromite process due to albumin.
Luther,	*J. C. S.*, lvi. 1039.	3 to 4 per cent. of nitrogen is oxidised to nitrate. Additional loss, 1½ per cent.
B. Dott,	*Pharm. Jour.* [3], xx. 793.	Calcium hypochlorite evolves total nitrogen promptly.
H. Smith,	*Pharm. Jour.*, [3], xxi. 294.	Modification of hypobromite process, using Allen's nitrometer filled with brine. Yield, 92 per cent.
H. Bartley,	*J. Amer. Chem. Soc.*, xii. 283.	Adds potassium bromide to sodium hypochlorite.

Year.	*Author.*	*Reference.*	*Remarks.*
1890-91	C. J. H. Warden, .	*Chem. News*, lxii. 287.	New apparatus for hypobr method.
1891	Heaton & Vesey, .	*Analyst*, xv. 106.	Rough apparatus for use of me practitioners.
1892	E. R. Squibb, . .	*Ephemeris*, iii. 1315.	Sodium carbonate with hypochl evolves all nitrogen promptly.
1893	W. Colquhoun, . .	*Chem. News*, lxvii. 123.	New form of apparatus and metho working hypobromite process
1895	H. J. H. Fenton, .	*Proc. Chem. Soc.*, No. 154.	Transformation of ammonium cya into urea.
1895	Walker & Hambly, .	*Proc. Chem. Soc.*, No. 154; *J. C. S.*, lxviii. 746.	Transformation of ammonium cya into urea, and reverse action.
1896	A. H. Allen, . .	*Proc. Chem. Soc.*, No. 160, Feb. 6, 1896.	Duggan's modification with additi potassium cyanate evolves t nitrogen.

The hypobromite solution employed for the decomposition of urea is prepared by dissolving 100 grammes of good caustic soda in 250 c.c. of water, and thoroughly cooling the liquid; from 20 to 25 c.c. measure of bromine is then added, and the resultant solutio preserved in a cool plac The reagent does not kee very well, in consequence the gradual occurrence the reaction : — $3NaBrO =$ $2NaBr + NaBrO_3$. Hence is preferable to prepare th solution, when required, b mixing 25 c.c. of the caust soda solution with 2·2 c.c. bromine. Dilution of th hypobromite reagent wit water, or the use of les bromine, does not greatl affect the results, but a larg excess of bromine is preju dicial.

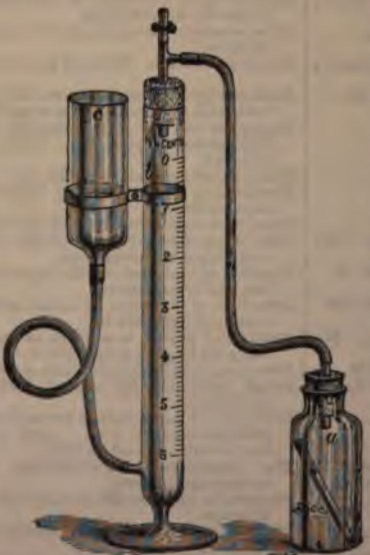

Fig. 9.—GERRARD'S UREOMETER.

Of the various forms apparatus which have bee proposed for effecting the r action and collecting th evolved nitrogen from urin that devised by A. W Gerrard (*Pharm. Jour.*, [3], xv. 464) is among the bes when no extreme degree of accuracy is aimed at. Gerrard apparatus (fig. 9) consists of a graduated tube, which is con nected with a second tube, serving as a reservoir, by means india-rubber tubing. The top of the graduated tube is closed by a caoutchouc stopper, through which passes a T-tube, one orifice of which is fitted with a short piece of india-rubber tubing closed by a clip, while the other communicates by a second piece of tubing with a bottle fitted with a perforated cork. In making the test, 25 c.c. of the hypobromite reagent should be poured into this bottle, and then a small test-tube containing 5 c.c. of the sample of urine cautiously placed in it in such a manner as to avoid any contact between the urine and the reagent. The bottle is now connected with the graduated tube in the manner shown in the figure, the clip opened, and water poured into the reservoir-tube until, on suitably adjusting its height, the water stands at the zero-point in the measuring-tube and at the same level in the reservoir, taking care that when this is effected, but little water remains in the latter tube.[1]

The clip is then closed, and the bottle is tilted in such a manner as to allow the urine to mix gradually with the hypobromite solution, the bottle being gently agitated to promote the evolution of gas, which commences immediately and is complete in a few minutes. After five minutes, or preferably ten, the water in the measuring-tube is brought to the same level with that in the reservoir-tube by lowering the latter, when the volume of gas is read off.[2] In Gerrard's apparatus the tube is so graduated as at once to show the percentage of urea contained in the urine.[3]

In the absence of any specially constructed apparatus for the estimation of urea, a suitable arrangement can be readily extemporised. Thus, if preferred, the nitrogen may be simply collected in a graduated tube filled with and standing over water, but it is more convenient to employ a graduated tube open at the upper end, which is drawn out so as to admit of being readily connected with the india-rubber tube attached to the evolution-flask. An inverted Mohr's burette, of a capacity of 50 c.c., immersed in a cylinder of water, answers very well. As long ago as 1877, A.

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[1] This can be effected by raising the reservoir, and is necessary to make room for the water displaced by the gas subsequently evolved.

[2] In case the temperature of the room is greatly different from 15·5° C. (=60° F.) the measuring-tube should be immersed in water at that temperature, but for ordinary purposes, and under ordinary conditions, this precaution is unnecessary.

[3] Gerrard's apparatus is so graduated as to allow for the loss of nitrogen which occurs under ordinary conditions, and hence gives correct results when non-saccharine urine is under examination. But when urine containing much sugar is under examination the correction described on page 272 should be applied.

Dupré (*Jour. Chem. Soc.*, xxxi. 534) recommended a tube of this kind, but furnished with a side-tube to which a clip was attached, which arrangement afforded some facilities in manipulating. These devices have all been superseded by the *nitrometer*, the employment of which for the purpose was first proposed by the author (*Jour. Soc. Chem Ind.*, iv. 179).[1]

Fig. 10 shows the upper part of a Lunge's nitrometer, the

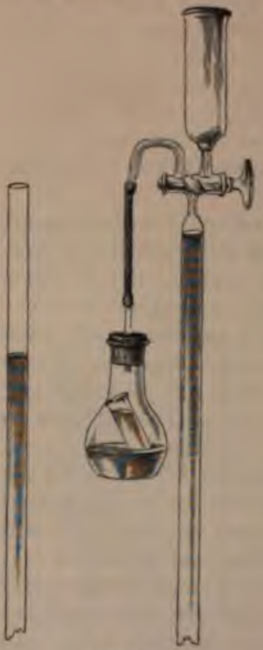

Fig. 10.

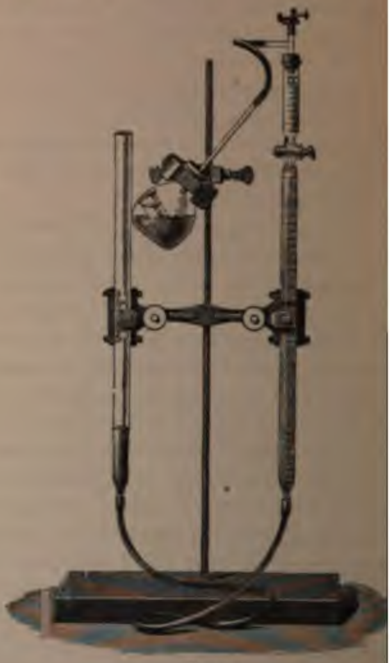

Fig. 11.

method of working with which is almost the same as wit Gerrard's form of apparatus. The hypobromite solution is place in the flask, the tube charged with the sample of urine is care fully introduced, the stopper firmly adjusted, and the india-rubbe leading-tube attached to the nose of the three-way tap. The ta is next turned, so as to allow the nitrometer-tube to communicate with the external air, and the open reservoir-tube raised until the liquid in the graduated tube just rises to the zero-point, whereon the tap is closed. The tap is next turned so as to connect the flask with the nitrometer-tube, while cutting off the external air. The urine is then allowed to come gradually into contact with the reagent in the manner already described. When the reaction is complete, the liquid in the reservoir-tube is brought to the level of that in the nitrometer, and the volume of gas read off.

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[1] Dupré's apparatus appears to have been the first ureometer in whic the principle of the nitrometer was adopted. The same principle was utilise in Gerrard's arrangement, the description of which was published a few month before the appearance of the author's paper on "New and little-know applications of the Nitrometer"; but the apparatus had been used an sketched by the author some years previously.

In the absence of a Lunge's nitrometer, the simpler form of apparatus devised by the author for assaying spirit of nitrous ether (*Pharm. Jour.*, [3], xv. 673), and known as "Allen's nitrometer" (fig. 11), will answer every purpose.

For all ordinary purposes, it is convenient and sufficiently accurate to fill the nitrometer with water, but mercury is sometimes used in research-work.

Another mode of using the nitrometer, and one which does not necessitate the use of an instrument provided with a three-way tap or equivalent arrangement, has also been described by the author (*Jour. Soc. Chem. Ind.*, iv. 179). For this purpose the nitrometer is filled to the tap with strong brine, and the tap closed. Five c.c. of the sample of urine[1] should then be placed in the cup by means of a pipette, and allowed to flow into the tube by opening the tap cautiously. The last traces are rinsed in by a few drops of water. Ten c.c. of the hypobromite solution, previously diluted with an equal measure of water, is next added through the tap. The greater part of the nitrogen is liberated as soon as the reagent comes into contact with the urine. When the reaction has somewhat abated, a clip is placed on the india-rubber connecting-tube and the nitrometer-tube vigorously shaken. When the reaction is completed, the clip may be removed, the liquids in the nitrometer- and reservoir-tubes brought to the same level, and the volume of gas observed. If the reading be rendered difficult from the formation of a persistent froth, this may be instantly destroyed by introducing a few drops of alcohol through the tap.

According to the great majority of observers, the reaction of cold hypobromite solution of the ordinary strength on pure solutions of urea results in the evolution of from 92 to 93 per cent. of the total nitrogen.[2] The suppressed nitrogen was found by H. J. H.

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[1] If the volume of gas evolved exceed 40 c.c., a smaller measure of urine should be employed.

[2] According to T. G. Wormley (*Chem. News*, xlv. 27), under favourable conditions the whole of the nitrogen of urea is evolved as gas. In the author's

Fenton to suffer conversion into cyanate.[1] On heating the liquid, some further evolution of gas occurs, but the theoretical production is never realised, and under some circumstances there is a tendency to error from evolution of oxygen. In presence of cane-sugar, a more complete evolution of the nitrogen occurs, and hence C. Méhu (*Bull. Soc. Chim.*, [2], xxxiii. 410; abst. *Jour. Chem. Soc.*, xxxviii. 681) has proposed always to add 10 parts of cane-sugar for each 1 of urea supposed to be present. Glucose induces a still more perfect evolution of the nitrogen, and consequently the evolution of gas on treating diabetic urine with hypobromite sometimes reaches 99 per cent. of the total ureal nitrogen present. Even in normal urine, the evolved nitrogen bears a greater proportion to the total amount present in the urea than is the case when pure solutions of urea are operated on. This fact is commonly attributed to the liberation of nitrogen from the uric acid, creatinine, and other urinary constituents, which, though present in but small amount relatively to the urea, are able to exert a sensible influence on the proportion of gas evolved. For ordinary purposes, the error due to this cause is wholly unimportant, and it has even been contended that, as the usual object of determining urea is to obtain a measure of the nitrogenous waste, all nitrogenised bodies of the urine should, as far as possible, be determined. Of course this is directly effected by determining the total nitrogen by Kjeldahl's process.

Méhu points out that the uric acid present in urine is rarely more than 2 per cent. of the urea, and the creatinine still less, and as these bodies only evolve a portion of their nitrogen when treated with hypobromite they are incompetent to produce the whole of the effect ascribed to them. He attributes the better yield of nitrogen obtained from normal urine to the extractive matter present, which acts more or less like sugar. Hence Méhu recommends the addition of sugar in every case, so as to ensure the evolution of practically the whole of the nitrogen in the gaseous state.

G. Esbach (*Bull. Soc. Chim.*, [2], xxiv. 632) states that pure glucose solution, whether boiling or not, evolves a small quantity of gas when treated with the hypobromite reagent, but that cane-

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experience, when operating in the ordinary manner, 92 per cent. of the total nitrogen is the highest yield for pure solutions of urea. Some German chemists add caustic soda to the urea solution before bringing it in contact with the hypobromite reagent, and by this means obtain 95 per cent. of the nitrogen as gas.

[1] Fauconier and Luther state that from 3½ to 4 per cent. of the total nitrogen is oxidised to nitrate.

gar yields no gas. He finds that in aqueous solutions of urea, e excess over the 92 per cent. normally evolved varies with the uantity and nature of the sugar added, the strength of the urea lution, and the composition, especially as to free alkali, of the hypo- romite reagent, the volume of gas being greater the more alkali here is present. For the proportions of glucose present in diabetic rine, the excess of nitrogen over 92 per cent. is sensibly propor- ional to the mass of the sugar, but this does not hold good for arge quantities. Urea *added* to a true diabetic urine is stated to volve only 92 per cent. of its nitrogen. Esbach concludes that ugar should not be added to urine before applying the hypobro- mite process.

The author has observed that glucose and cane-sugar occasion reat evolution of heat, and probably this phenomenon is one of he causes of the higher results obtained in presence of sugar. auconier states that glucose prevents loss by formation of nitrate, ut that cane-sugar has no similar action.

Jacobi (*Zeitschr. anal. Chem.*, xxiv. 307) suggests that the igher proportion of nitrogen yielded by diabetic urine may be ue to the presence of aceto-acetic ether, which is often present in vanced glycosuria. He found by experiment that the addition 5 per cent. of aceto-acetic ether to pure solutions of urea caused e evolution of the total nitrogen as gas on treatment with hypo- omite.

In interpreting the results obtained by any of the foregoing odifications of the hypobromite method of estimating urea, it is un- cessary for ordinary purposes to take into account the barometric essure, tension of aqueous vapour, or temperature at the time of aking the experiment. By a fortunate coincidence, the increase in e volume of the gas, due to its being measured in a wet condition d at a temperature of 18° C. (=65·4° F.), almost exactly com- nsates for the $7\frac{1}{2}$ to $8\frac{1}{2}$ per cent. of the total nitrogen ordinarily ppressed in the reaction. Thus Russell and West (*Jour. hem. Soc.*, xxvii. 749) found that 0·100 gramme of urea gave 37·1 c.c. of moist nitrogen at a temperature of 65° F. (=18·3°). This, if dry, and at the standard pressure and temperature, ould have measured 34·05 c.c., whereas the *total* nitrogen con- ined in the same amount of urea would measure 37·14 c.c. under e standard conditions, or nearly the volume actually obtained the ordinary temperature. Hence 37·1 c.c. of gas, measured nder the ordinary conditions of experiment, may be taken to epresent 0·1 gramme of urea. Thus, if the cubic centimetres of as evolved be multiplied by $\frac{1000}{371}=2{\cdot}7$ (or, more exactly, 2·696) he product will be the number of milligrammes of urea in the

volume of urine employed; or, if 5 c.c. of urine were take
the experiment, the volume of gas evolved, multiplied by 0
(or, more exactly, 0·0539), will be the grammes of urea (so-c
"percentage") in 100 c.c. of the urine. The percentage
found, multiplied by 4·375, will be the number of grains of
contained in 1 fluid ounce of the urine.

If preferred, the above calculations can be combined, and
grains of urea per fluid ounce of the sample found at one
multiplying the measur
gas evolved from 5 c.
the sample by 0·236
more exactly, 0·23585).

The foregoing factors
intended for use with
saccharine urine. As
evolution of the nitroge
urea is more complete in
presence of sugar than i
absence, the results yie
when diabetic urine is tr
with hypobromite shoul
multiplied by the factor
to obtain more correct re
A plan sometimes practis
to add some simple syru
honey (from 3 to 5 c.c.) t
urine in the sample-
Under these circumsta
40 c.c. of nitrogen (ag
37·1 c.c. in the absenc
sugar) are said to be yi
by 0·1 gramme of urea.
in the author's experienc
increase in the yield of
is very variable, the nitr
evolved being from 92 to 99 per cent. of the total, and
method far less satisfactory than that next to be described.

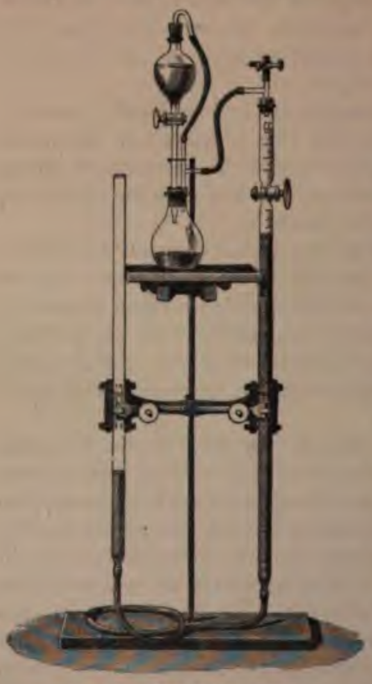
Fig. 12.—ALLEN'S UREOMETER.

The author's experience of the ordinary forms of the hypobro
method of estimating urea, under a great variety of condit
and with the utmost care to obtain accurate results, leads hi
the conclusion that the process in its ordinary form is b
means so accurate, nor even so constant in its indication
is commonly supposed. Nevertheless, the hypobromite proce
undoubtedly a method of great practical utility, and one w

is capable of doing excellent service in the hands of intelligent operators.

6. *Allen's modified Hypobromite Process.* — A considerably greater proportion of the nitrogen of urea is evolved as gas, and the process thereby correspondingly improved, if the bromine be added to a previously-made mixture of the caustic soda and urea solutions. For this purpose the author has found the modified form of apparatus shown in fig. 12 (page 272) to be the most convenient. In this arrangement, a separating funnel is substituted for the sample-tube generally used. 25 c.c. of a 40 per cent. solution of caustic soda should be placed in the flask, and 5 c.c. of the urine or other solution of urea added. The cork is then adjusted, and a solution of 2 c.c. of bromine in 16 c. of a 20 per cent. aqueous solution of potassium bromide added radually from the tapped separator, the flask being repeatedly gitated. The evolution of nitrogen occurs very promptly, the ield being from 95 to 98 per cent. of the total, as against 91 o 92 per cent. in the ordinary process.[1] The evolution of nitrogen s generally complete with considerably less than the volume of he bromine solution above prescribed.

In a recent paper (*Jour. Chem. Soc.*, 1895, lxvii. 746), Walker nd Hambly have described some suggestive researches on the ansformation of ammonium cyanate into urea. They find that he reaction is never complete. They further prove, as previously uggested by Fenton,[2] that the reverse reaction occurs when an queous solution of urea is boiled, and that the well-known formaon of silver cyanate when urea is boiled with silver nitrate (see age 254) is really due to this cause. It follows that a solution ontaining both ammonium cyanate and urea ultimately arrives at condition of equilibrium, which is upset if ammonium sulphate r potassium cyanate be added to the solution, the urea in each ase being rendered more stable.

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[1] The proposal to add the bromine to the mixed solution of urea and austic soda appears to be due to J. R. Duggan (*Amer. Chem. Jour.*, iv. 7 ; abst. *Jour. Chem. Soc.*, xlii. 778), who states that fully 99 per cent. of the real nitrogen is evolved as gas if 5 c.c. of urine be first mixed with 20 c.c. f a solution of 20 grammes of caustic soda in 100 c.c. of water, and 1 c.c. of romine gradually added. Working with these quantities, the author has een unable to obtain so high a yield of gas as 99 per cent., but the reversal f the operation, as proposed by Duggan, is undoubtedly a valuable improveent on the ordinary mode of working.

[2] Fenton inferred the existence of a reverse reaction from the fact that onversion of ammonium cyanate into urea was never complete. He further ound that ammonia and cyanate were formed when a solution of urea was reated with caustic potash and exposed over sulphuric acid.

Reasoning from these results, it appeared to the author to be probable that the incomplete evolution of nitrogen in the hypobromite process might be due to a reversion of a portion of the urea to the condition of cyanate; especially as H. J. H. Fenton had shown that cyanate was a product of the action of alkaline hypochlorites on urea, and W. Foster had extended the observation to the action of the ordinary hypobromite reagent, and showed the 8 per cent. of suppressed nitrogen to exist in the solution in the form of cyanate. Fenton further found that cyanates evolved no gas when treated with the hypobromite reagent, and the author has fully confirmed this observation. Hence it appeared possible that by adding a sufficiency of potassium cyanate to the urea solution before treating it with the hypobromite, the reversion of the urea to cyanate might be entirely prevented. Experiment has proved this conjecture to be correct. An addition of 0·250 gramme of pure potassium cyanate to the solution of 0·100 gramme of urea in 5 c.c. of water raises the evolved nitrogen from 91 to nearly 97 per cent. (corrected). When the modified form of apparatus shown in fig. 12 is employed, and the cyanate added to the solution of urea, allowed to dissolve, and followed by caustic soda, the bromine solution being then run in gradually, as described on page 273, still better results are obtained. Under these conditions, the yield of nitrogen is from 99·8 to 100·0 per cent. (corrected). Hence the addition of potassium cyanate, to the extent of two to three times the amount of the urea present, effects a complete evolution of the nitrogen in the form of gas, and prevents the irregularities and uncertainty attaching to the hypobromite process in its ordinary form.[1] It is far preferable to

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[1] The experiments on which this recommendation is based have only been completed on the eve of going to press, but may be relied on to substantiate the claim made in the text. Operating as there described on 5 c.c. of a 2 per cent. solution of pure urea, A. R. Tankard has obtained, in the author's laboratory, an evolution of 99·8, 100·0, 100·0, 99·8, and 100·1 per cent. of the total nitrogen.

Potassium cyanate is unstable and, unless freshly-prepared, is apt to contain ammonia. Hence it is desirable to make a blank experiment, and deduct any nitrogen evolved in the absence of urea from the volume liberated in its presence.

The use of silver cyanate, with or without simultaneous addition of sodium chloride, was found less satisfactory than the addition of the potassium salt.

It appeared probable that the reaction of the hypobromite reagent on potassium cyanide would result in the formation of cyanate, in which case potassium cyanate could be conveniently extemporised *in situ*. Experiment showed that on adding potassium cyanate to the hypobromite reagent great evolution of heat occurred, and some cyanate was formed, but the product had not the same effect as previously prepared potassium cyanate. The

the addition of sugar or glucose, which increases the yield of gas to a variable extent.

When the modified method is employed, and potassium cyanate added to the urea solution, 1 c.c. of nitrogen, measured moist and at the ordinary pressure and laboratory temperature, represents 0·0025 gramme of urea. Hence if 5 c.c. of urine be employed, each c.c. of gas evolved represents 0·05 per cent. of urea in the sample.

Nitrogen evolved by hypobromite from various compounds.—The following table shows the results recorded on treating various nitrogenised compounds with the hypobromite reagent:—

Substance.	Formula.	Percentage of total Nitrogen evolved as gas with hypobromite and excess of caustic soda.	Observer.
[1]Acetamide, ...	C_2H_5NO.	cold, none; hot, 3½.	Foster; Allen. A. H. Allen.
Allantoin, ...	$C_4H_6N_4O_3$.	50.	P. Malebra (*J. C. S.*, l. 583).
Ammonium aspartate, .	$C_4H_{10}N_2O_4$.	49, on heating.	Allen and Tankard.
Ammonium carbamate, .	$N_2H_6CO_2$.	100.	Fenton (*J. C. S.*, xxxv. 12).
Ammonium sulphate, .	$N_2H_8SO_4$.	93–100.	Fenton; Foster; Allen.
Asparagine, ...	$C_4H_8N_2O_3$.	none.	A. R. Tankard.
Biuret, ...	$C_2H_5N_3O_2$.	67.	Fenton (*J. C. S.*, xxxv. 12).
Creatine, ...	$C_4H_9N_3O_2$.	67.	Hüfner.
Creatinine, ...	$C_4H_7N_3O$.	50.	A. R. Tankard.
Glycocine, ...	$C_2H_5NO_2$.	none.	Allen and Tankard.
Guanidine, ...	CH_5N_3.	67.	Fenton (*J. C. S.*, xxxv. 12).
Hippuric acid, . . .	$C_9H_9NO_3$.	none.	Allen and Tankard.
[2]Oxamide, ...	$C_2H_4N_2O_2$.	75.	Foster (*J. C. S.*, xxxiii. 472; xxxv. 119).
Potassium cyanate, . .	KCNO.	none.	Fenton (*J. C. S.*, xxxiii. 300; xxxv. 12); Allen.
Potassium ferrocyanide,	$K_4FeC_6N_6$.	33–50.	Foster (*J. C. S.*, xxxv. 123).
Urea, ...	CH_4N_2O.	91 to 92.	Fenton; Foster; Allen; &c.
Urea, with addition of potassium cyanate,	..	96·5 to 97·0.	A. H. Allen.
Do. do., by modified process (page 273),	..	100.	Allen and Tankard.
Uric acid, ...	$C_5H_4N_4O_3$.	variable, 46 to 85.	Allen and Tankard.
Uric acid, ...	..	65.	Russell and West.

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author proposes to investigate the reaction, which does not appear to have been previously studied.

[1] Foster (*Jour. Chem. Soc.*, xxxv. 119) states that acetamide begins to be attacked at 80°, but yields only a portion of its nitrogen in the gaseous state. The author finds that no gas at all is evolved in the cold, and even when the mixture of hypobromite and acetamide is kept at a boiling heat for some minutes the gas evolved is insignificant, representing only about 3 per cent. of the total nitrogen.

[2] Foster found that in the cases of urea, oxamide, and potassium ferrocyanide, the suppressed nitrogen was present in the solution in the form of cyanate. In the last case, no perceptible change occurred in the cold, but on boiling a ferrate was also formed and oxygen evolved.

7. *Determination of Urea by Hypochlorites.*—The hypobromit method of determining urea may be considered as a modificatio of the process originally devised by E. R. Davy, in which th urea was brought in contact with a solution of bleaching powder. D. B. Dott and other chemists have expressed a preference for the same reagent, while E. R. Squibb and others have employed sodium hypochlorite. Fairly good results are obtainable with mixture of sodium carbonate with sodium hypochlorite, such results from the precipitation of a solution of bleaching powde with a large excess of sodium carbonate; but if caustic soda b substituted for the carbonate very erratic results are liable to b obtained. By employing a very large excess of both sodium hyp chlorite and of caustic soda, H. J. H. Fenton obtains an approxi mate yield of 50 per cent. of the total nitrogen in the form gas, the remainder being converted into cyanate. A series of experi ments made in the author's laboratory with the view of verifyin this curious fact have shown that a rigid adherence to certain con ditions is essential to ensure success.[1]

In the author's experiments, bleaching powder was treated wit four times its weight of cold water, the liquid filtered throug asbestos, and the filtrate treated with solid sodium carbonate slight excess. It was then again filtered through asbestos. Th caustic soda was a solution of 100 grammes in 250 c.c. of wate and the urea was employed in 2 per cent. solution, so that 5 c. represented 0·100 gramme of urea. The mixed hypochlorite an caustic soda solutions were placed in the flask shown in fig. 12, page 272, and the urea solution introduced in a sample-tube, as in fig. 11, page 268. The contents of the tube were then caused to mix with the hypochlorite by inclining the flask, which was shaken at intervals as long as gas continued to be evolved. This occurred somewhat sluggishly, but was usually complete in half an hour. In some experiments the action was allowed to continue for several hours. The hypobromite reagent (or its equivalent) was then allowed to run into the flask through the tap of the separating funnel, and the volume of nitrogen gas evolved again observed. In all cases the amounts of the reagents were many times those theoretically required. Fenton found the suppressed nitrogen to be converted into cyanate, and hence in his experiments no further evolution of gas occurred on subsequently treating the liquid with the hypobromite reagent. The same fact was observed by the author in certain experiments; but the percentages of the total nitrogen evolved by the action of the hypochlorite were very variable, apparently decreasing irregularly with an increase in the quantities of the reagents. In some experiments a notable quantity of gas was evolved on treating the solution with hypobromite after the completion of the hypochlorite reaction. Such evolution of nitrogen is regarded by Fenton as characteristic of a carbamate. Hence the inference from the author's experiments is that on treating urea with a hypochlorite solution and caustic soda, a variable proportion (from 27 to nearly 100 per cent.) is completely split up with evolution of the nitrogen as gas; a second variable quantity (varying from *nil* to 50 per cent.) is converted into carbamate; while a third reverts to the condition of cyanate.[1] Nitrate is alleged to be formed when hypobromite is employed, but is stated by Fenton to be absent from the products of the action of hypochlorite on urea.

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[1] Fenton lays much stress on the mode of preparation of the hypochlorit He passes chlorine through a 10 per cent. solution of caustic soda until it nearly saturated, and mixes the product with an equal measure of a stro solution of caustic soda (1 in 3). Fenton employs at least 80 c.c. of th mixture for decomposing 0·050 gramme of urea, the solution of which introduced in a sample-tube. Vigorous and repeated agitation of the co taining flask or bottle is essential. Under these conditions the reaction found by Fenton to be complete in 30 to 40 minutes, and no further evoluti of nitrogen occurs on subsequently adding a bromide or hypobromite, th proving that no carbamate has been formed. The nitrogen evolved is fr 48 to 60 per cent. of the total, so that the reaction has rather a scienti interest than one on which can be based a method for the accurate determin tion of urea; but Fenton suggests that it may be applied to the approxima estimation of urea in presence of ammonia and of carbamates.

Fenton (*Jour. Chem. Soc.*, xxxv. 12) gives the following as the perce age of the total nitrogen evolved from various nitrogenised compounds treatment with caustic soda, and a hypochlorite instead of hypobromite Ammonium carbamate, 50 (the suppressed nitrogen remaining in the liqu as sodium carbamate); biuret, 33; potassium cyanate, none; guanidin 67; and urea, 50 per cent. of the total contained nitrogen.

Separation of Urea from Ammonia.—Ammoniacal compounds, when treated with the hypobromite reagent, yield their nitrogen in the form of gas as promptly and perfectly as does urea. Hence the volume of nitrogen evolved when urine is submitted to the process includes that existing as ammonia as well as that present as urea; but as urinary ammonia usually has its origin in preexisting urea, it is rarely necessary to determine it separately. When this is desired, it may be effected by titrating 50 c.c. of the urine with litmus and normal sulphuric acid, each c.c. of which represents 0·017 gramme of ammonia or 0·030 of urea.

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[1] The larger the quantities of the reagents used, the less appeared to be the tendency to form carbamate, and the nearer was the approximation to Fenton's reaction. But the fact that a carbamate is apparently formed under certain conditions shows that the reaction is not yet fully understood.

This process, of course, only estimates ammonia existing as carbonate or in a free state. The determination of the total ammonia existing in urine may be effected by precipitation with platinic chloride, distillation of the chloroplatinate with soda, and titration of the ammonia in the distillate. A simpler plan is as follows :—Render 100 c.c. of the urine exactly neutral to litmus. Add 10 c.c. of normal caustic soda, and boil the liquid in a capacious flask as long as the steam has an alkaline reaction.[1] The residual liquid is then titrated with decinormal acid and litmus. The loss of alkalinity represents the ammonia volatilised. Any decomposition of urea which may occur does not affect the results, since urea is neutral to litmus.

In certain physiological researches it is important to distinguish between the nitrogen eliminated in the form of urea and that existing as ammoniacal compounds, and to determine both forms with the greatest attainable accuracy. In such cases, Mörner and Sjöquist (*Zeits. anal. Chem.*, xxx. 388) determine the urea by the following method, the value of which is confirmed by E. Bödtker (*Zeits. physiol. Chem.*, xvii. 140). Five c.c. measure of the urine is mixed in a flask with an equal measure of a saturated solution of barium chloride containing 5 per cent. of barium hydroxide. To this is added 100 c.c. of a mixture of two measures of absolute (97 per cent.) alcohol with one measure of ether, and the whole left in the closed flask for twenty-four hours. The liquid is then filtered, and the precipitate washed with the ether-alcohol. The filtrate and washings are distilled (assisted by bubbling air through the liquid) at a temperature not exceeding 55° C., until reduced to a volume of 25 c.c. A little water and calcined magnesia are then added, and the liquid distilled until the vapours are no longer alkaline. The liquid remaining in the flask is treated with a few drops of concentrated sulphuric acid, and the urea estimated by Bunsen's or Kjeldah method.

Determination of Urea in Blood and Tissues.

When it is desired to determine the proportion of urea prese in animal products other than urine, it is necessary to remo various interfering substances. A convenient plan, in the case blood or tissues, is to treat the substance with alcohol in sufficie quantity to coagulate the proteids, and evaporate the alcohol extract. The residue is in many cases sufficiently pure to allo of the separation of the urea as oxalate, or of its determination by hypobromite. M. Kaufmann treats the residue with Millon's reagent (a solution of nitrate of mercury containing nitrous acid) over mercury, measuring the nitrogen and carbon dioxide evolved. Other extractives do not interfere, and the results are said to be very satisfactory. B. Schöndorff (*Pflüger's Archiv.*, 1895, lxii. 57) treats the finely-divided substance with alcohol, acidifies the filtered liquid with acetic acid, evaporates to dryness, takes up the residue with alcohol, and again evaporates the filtered liquid to dryness. The residue is extracted with hot water, and the solution treated with a solution of phospho-tungstic acid containing 10 per cent. of hydrochloric acid of 1·12 sp. gravity. This reagent completely precipitates creatinine (not creatine), xanthine, guanine, and uric acid; but not urea or the amido-bases. These latter compounds, however, do not give off nitrogen when heated to 150° with phosphoric anhydride, and yield only insignificant traces of carbon dioxide when heated under pressure at 150° with an alkaline solution of barium chloride. Urea, on the other hand, yields all its nitrogen as gas when heated to 150° with phosphoric anhydride, and is completely decomposed into ammonia and carbon dioxide at the same temperature by alkaline barium chloride. Hence, Schöndorff renders the filtrate from the precipitate produced by phospho-tungstic acid alkaline with powdered lime, and determines in the filtered liquid the total nitrogen by Kjeldahl's process; the nitrogen evolved by treatment at 150° with phosphoric anhydride, and the carbon dioxide produced by heating with alkaline barium chloride, which hould give one molecule of carbon dioxide for two of ammonia produced in the previous process.

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[1] A troublesome froth is apt to form on first heating, but it subsides aft cautious ebullition for a few minutes.

Pekelharing (*Jour. Chem. Soc.*, xxix. 775) considers Bunsen's method of estimating urea objectionable. For the estimation of urea n the blood, liver, &c., he extracts the liquid or viscous matter, nd coagulates albumin, by heating with faintly acidulated water. The filtered liquid is precipitated by lead acetate, filtered, freed rom excess of lead by sulphuretted hydrogen, again filtered, and evaporated to a small bulk. The concentrated liquid is acidified by acetic acid and the urea precipitated by mercuric nitrate. (See also P. Picard, *Compt. rend.*, lxxxiii. 991; abst. *Jour. Chem. Soc.*, xxxi. 486.)

Para-phenetol-carbamide. Dulcine. Sucrol.

$$C_9H_{12}N_2O_2\text{; } i.e.,\ CO\left\{\begin{array}{l}NH.C_6H_4.O(C_2H_5)\\NH_2\end{array}\right.$$

This substance has a pure sweet taste, about 200 times as intense as that of cane-sugar, and is now employed as a substitute for benzoic sulphinide ("Saccharine," Part i. page 26) in cases where the use of sugar is objectionable.

Dulcine was first prepared by the reaction of paraphenethidine hydrochloride (Part ii. page 81) with potassium cyanate, but has been subsequently obtained by the reaction of paraphenethidine with carbon oxychloride, and treatment of the resultant chloro-compound with ammonia[1]:—

$$a.\quad 2NH_2.C_6H_4.OC_2H_5 + COCl_2 = NH_2.C_6H_4.OC_2H_5,HCl + COCl.NH.C_6H_4.OC_2H_5$$

Paraphenethidine.

$$b.\quad COCl.NH.C_6H_4.OC_2H_5 + 2NH_3 = NH_4Cl + NH_2.CO.NH.C_6H_4.OC_2H_5$$

Dulcine.

Dulcine has also been prepared by the reaction of paraphenethidine with aniline, when it is produced together with diparaphenetol carbamide. The taste of the latter compound is not sweet, but by heating for several hours to 160° with an equivalent quantity of urea it is converted into the monophenetol-derivative (dulcine), according to the equation :—

$$CO{:}(NH.C_6H_4.OC_2H_5)_2 + CO(NH_2)_2 = 2(NH_2.CO.NH.C_6H_4.OC_2H_5).$$

Diparaphenetol-carbamide. Urea. Dulcine.

Dulcine is also produced by heating the diphenetol-urea with ammonium carbamate or commercial carbonate of ammonium.

Dulcine forms colourless or yellowish shining needles, whi melt at 173° to 174° (at 160°, Morpurgo). It is soluble about 800 parts of cold or in 50 of boiling water, and dissolves 25 parts of rectified spirit. It is also soluble in ether and benzene. When pure it dissolves in concentrated sulphuric ac without coloration. When boiled with water, dulcine gradually decomposes into ammonium carbonate and diparaphenetol-carbamide.

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[1] A solution of paraphenethidine in benzol is gradually added to a 20 p cent. solution of carbon oxychloride in the same solvent. After standing f an hour or so, the liquid is filtered, and the filtrate treated with ammon gas or shaken with a strong solution of ammonia. The ammonium chloride filtered off and the filtrate evaporated, the residue washed with cold wate and the dulcine recrystallised from boiling water.

If concentrated solutions are employed in the foregoing process dipar phenetol-carbamide is also formed. According to F. v. Heyd in operating on a large scale, the carbonyl chloride compound is not form or only in small amount, the reaction taking place with formation of para ethoxyphenyl isocyanate, $CO{:}N.C_6H_4.OEt$, which body yield dulcine on treatment with ammonia.

Dulcine is also obtained by heating paraphenethidine with urethane or wit acetyl-urea.

On adding fuming nitric acid to a fragment of solid dulcine a violent reaction occurs, and the substance dissolves with orange-red coloration. On evaporating the liquid to dryness at 100°, an orange-yellow resinous substance is obtained, which is soluble in ether, chloroform, or alcohol. If this resinous product be triturated with a mixture of equal parts of phenol and strong sulphuric acid the colour changes to a blood-red, which is permanent for a considerable time.

For the detection of dulcine in wine, beer, &c., G. Morpurgo treats the suspected liquid with 5 per cent. of lead carbonate, evaporates on a water-bath to a thick paste, and treats the residue several times with strong alcohol. The alcoholic solution is evaporated to dryness, and the residue extracted with ether. On evaporating the ether, a residue is obtained of nearly pure dulcine, which may be recognised by its sweet taste and by the following reaction. The residue is warmed with two drops of phenol and two drops of concentrated sulphuric acid, the resultant reddish-brown syrup rinsed into a test-tube with a few c.c. of water, and he liquid cooled. Ammonia or caustic soda is then poured autiously on to the surface, when the production of a blue or iolet-blue zone at the junction of the two layers will indicate he presence of dulcine.

Dulcine does not appear to affect the digestive action of ptyalin r pepsin, and has no antiseptic properties. Considerable doses re stated to produce no distaste or injurious effects, even when ong continued.

IMIDO-BASES.

The imido-bases are distinguished by containing the group —NH, otherwise than as a link in a closed chain, as it exists in anthine, uric acid, &c. Indole and skatole do not come within his description, and in other respects present marked differences rom the true imido-bases; but they are conveniently classed nd considered therewith. In some cases, as in that of guanidine (page 282), the imido-bases also contain one or more mido-groups. The members of the group have in some cases onsiderable interest, and certain of them are important constituents of meat-juice and other animal products; but none f them have hitherto received any practical application in an isolated state. The chief members of the class may be thus formulated :—

GUANIDINE.

$$\mathrm{NH{:}C}\left\{\begin{matrix}\mathrm{NH_2}\\ \mathrm{NH_2}\end{matrix}\right.$$

METHYL-GUANIDINE.

$$\mathrm{NH{:}C}\left\{\begin{matrix}\mathrm{NH_2}\\ \mathrm{NH.Me}\end{matrix}\right.$$

GLYCOCYAMINE.[1]
(Guanido-acetic acid.)

$$\mathrm{NH{:}C}\left\{\begin{matrix}\mathrm{NH_2}\\ \mathrm{NH.CH_2.COOH}\end{matrix}\right.$$

CREATINE.
(Methyl-Glycocyamine.)

$$\mathrm{NH{:}C}\left\{\begin{matrix}\mathrm{NH_2}\\ \mathrm{NMe.CH_2.COOH}\end{matrix}\right.$$

GLYCOCYAMIDINE.[2]

$$\mathrm{NH{:}C}\left\{\begin{matrix}\mathrm{NH.CO}\\ \mathrm{NH.CH_2}\end{matrix}\right\}$$

CREATININE.
(Methyl-Glycocyamidine.)

$$\mathrm{NH{:}C}\left\{\begin{matrix}\mathrm{NH.CO—}\\ \mathrm{NMe.CH_2}\end{matrix}\right\}$$

INDOLE.

$$\mathrm{NH{:}}\left\{\begin{matrix}\mathrm{CH{:}CH}\\ \mathrm{C_6H_4—}\end{matrix}\right\}$$

SKATOLE.
(Methyl-Indole.)

$$\mathrm{NH{:}}\left\{\begin{matrix}\mathrm{CH{:}CMe}\\ \mathrm{C_6H_4—}\end{matrix}\right\}$$

Guanidine. Imido-urea.

$$\mathrm{CH_5N_3};\ i.e.,\ \mathrm{NH{:}C}\left\{\begin{matrix}\mathrm{NH_2}\\ \mathrm{NH_2}\end{matrix}\right.$$

Guanidine has not been detected in any animal tissue or fluid, but has been isolated from vetch-seedlings. It has been obtained by the direct oxidation of proteids, and yields urea by boiling with baryta-water or dilute acids. Guanidine is the chief product of the action of oxidising agents on guanine, and may be regarded as a connecting link between creatine and the xanthine bases.

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[1] **GLYCOCYAMINE** is obtained when glycocine is boiled with guanidine carbonate in aqueous solution, ammonia carbonate being simultaneously formed. It forms transparent needles, sparingly soluble in cold water, but readily on boiling, and insoluble in alcohol. Boiled with cupric acetate it gives microscopic crystals containing $\mathrm{Cu(C_3H_6N_3O_2)_2}$. According to A. B. Griffiths, glycocyamine occurs in putrid flesh.

Propyl-glycocyamine is stated by A. B. Griffiths to occur in the urine of patients suffering from mumps. It is described as a white, neutral solid, causing nervous excitement, convulsions, and death.

[2] **GLYCOCYAMIDINE** hydrochloride is obtained when glycocyamine hydrochloride is heated to 160°. On boiling the product with water and lead hydroxide the free base is obtained in laminæ of alkaline reaction, which have a bitter taste, are poisonous, and very soluble in water. Glycocyamidine forms a compound with zinc chloride which crystallises in needles closely resembling the corresponding compound of creatinine. According to A. B. Griffiths, glycocyamidine occurs in the urine of persons suffering from measles.

Guanidine has been obtained synthetically by several methods, among which may be mentioned the reaction of cyanamide with ammonia:—$CN.NH_2 + NH_3 = NH{:}C(NH_2)_2$. In practice, ammonium chloride is heated with an alcoholic solution of cyanamide.

Guanidine may be conveniently prepared by heating dry ammonium thiocyanate to 180°–190° C. for twenty hours. A portion of the thiocyanate is isomerised into thiourea, which reacts with the undecomposed ammonium thiocyanate to yield a product consisting mainly of guanidine thiocyanate. This is purified by crystallisation from water or alcohol, and the solution of 100 parts mixed with the solution of 58 parts of potassium carbonate, and evaporated to dryness. From the residue, the potassium thiocyanate is removed by treatment with alcohol, and the guanidine carbonate recrystallised from water. From this salt free guanidine is obtained by dissolving it in the calculated quantity of dilute sulphuric acid and adding an equivalent amount of baryta-water. On evaporation *in vacuo* over sulphuric acid, the guanidine is obtained in deliquescent crystals.

Guanidine is a strongly alkaline, crystalline substance having a caustic taste. It is readily soluble in water and alcohol. On exposure to air it deliquesces and absorbs carbon dioxide with conversion into the carbonate.

When boiled with baryta-water, guanidine yields ammonia and urea, thus:—$NH{:}C(NH_2)_2 + H_2O = NH_3 + CO(NH_2)_2$. The urea further splits up into ammonia and carbon dioxide. With hot concentrated acids and alkalies these are the sole products.

Guanidine is a monovalent base which forms a series of crystallisable salts with acids. *Guanidine nitrate*, CH_5N_3,HNO_3, forms crystalline plates which are sparingly soluble in water. B_2,H_2PtCl_6 is sparingly soluble in absolute alcohol.

Many salts of guanidine, including the nitrate, sulphate, carbonate, and hydrochloride, give with Nessler's reagent a white or faintly yellowish precipitate, at first flocculent and bulky, but collecting together after a time. E. Schulze (*Ber.*, xxv. 661) describes the reaction as very delicate; a 0·05 per cent. solution giving an appreciable precipitate, while even a 0·01 per cent. solution is rendered turbid.

Guanidine is not precipitated by lead acetate, but is separated very completely by phospho-tungstic acid, which reagent is employed by E. Schulze for its isolation from plant-substances.[1]

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[1] Vetch-seedlings which had grown for three weeks in the dark were dried, powdered, and digested with rectified spirit. The extract was filtered, distilled, the residue treated with water and some tannin, and then precipitated by lead acetate. The filtered liquid was precipitated with phospho-tungstic

Guanidine may be conveniently purified and determined by precipitating the solution of one of its salts by an aqueous solution of picric acid. Guanidine picrate requires 2600 parts of cold water for solution, and is only sparingly soluble in alcohol and ether. It crystallises in very characteristic forms, does not melt at 280°, but burns at a higher temperature. It does not detonate when struck.

When equivalent weights of guanidine and phenol are dissolved in hot alcohol, triphenyl-guanidine is formed, and on adding picric acid to a solution of this compound, the corresponding picrate, $CH_2Ph_3N_3,C_6H_3(NO_2)_3O$, is obtained as a precipitate of slender needles, melting at 208°, and requiring about 12,000 parts of cold water for solution. Guanidine aurichloride, $CH_5N_3,HAuCl_4$, forms long yellow needles difficultly soluble in water.

On treating guanidine carbonate with a solution of sodium hypobromite, as described on page 268, it is stated by H. J. H. Fenton that two-thirds of the nitrogen is evolved in the gaseous state, while the rest is converted into cyanate,[1] according to the equation :—$NH{:}C(NH_2)_2+O_3=HCNO+2H_2O+N_2$.

Guanidine forms an insoluble compound with mercuric oxide, which fact is utilised by Gergers and Baumann (*Pflüger's Archiv.*, xii. 205) for the isolation of the base from urine.[2]

Guanidine has marked poisonous properties. In dogs it produces paralysis, convulsions, vomiting, and difficult breathing; in frogs, muscular twitchings, paralysis, and (with a dose of 0·050 gramme) death.

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acid, the precipitate washed with dilute sulphuric acid, and decomposed with cold lime-water. The filtered liquid was freed from lime by carbon dioxide, the filtrate neutralised with nitric acid, and concentrated on the water-bath. On cooling, guanidine nitrate crystallised out, 1 gramme being obtained from 3 kilogrammes of vetch-seedlings (*Ber.*, xxv. 658).

[1] This reaction is remarkable in view of the fact that when gently warmed with a caustic alkali guanidine is decomposed into ammonia and urea, each of which yields nearly all its nitrogen in the form of gas when treated with hypobromite.

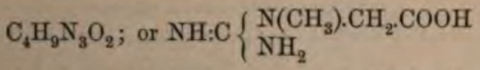
[2] The urine is precipitated with baryta-water, the filtrate neutralised by hydrochloric acid and evaporated to a syrup at 100°. The residue is exhausted with alcohol, the filtered liquid evaporated, and the residue taken up by a little water. The solution is shaken with freshly-precipitated mercuric oxide, and allowed to stand for two days in a warm place, when the precipitate is filtered off, treated with hydrochloric acid, and the mercury precipitated by sulphuretted hydrogen. The filtered liquid is evaporated and the residue dissolved in absolute alcohol. The solution is treated with platinic chloride, the precipitate of ammonium chloroplatinate removed by filtration, and the filtrate evaporated to a small bulk, when on long standing guanidine chloroplatinate crystallises out.

METHYL-GUANIDINE, $NH{:}C(NH_2).NH(CH_3)$, is produced by boiling creatine with mercuric oxide, or with dioxide of lead and dilute sulphuric acid. It has been isolated by Brieger from putrefying horse-flesh, and has been found in other decomposing animal matters.[1]

Methyl-guanidine forms a strongly alkaline, deliquescent, crystalline mass, which evolves ammonia and methylamine when boiled with caustic alkali. B,HCl crystallises in needles insoluble in alcohol. $B,HAuCl_4$ forms rhombic crystals, melting at 198°, very sparingly soluble in cold water, but readily dissolved by alcohol or ether. Methyl-guanidine picrate, when first precipitated, forms a resinous mass, which, by boiling with water, is converted into needles melting at 192° and soluble in boiling absolute alcohol.

Methyl-guanidine has marked poisonous properties, the symptoms observed being rapid respiration, mydriasis, paralysis, convulsions, and death. Brieger found it to produce choleraic symptoms.

Creatine. Methyl-glycocyamine. Methyl-guanidine-acetic Acid.



$$C_4H_9N_3O_2;\ \text{or}\ NH{:}C\left\{\begin{array}{l} N(CH_3).CH_2.COOH \\ NH_2 \end{array}\right.$$

Creatine has been obtained synthetically by heating sarcosine with an alcoholic solution of cyanamide, thus :—

$$CH_2NHMe.COOH + CN.NH_2 = NH{:}C(NH_2).(NMe).CH_2.COOH.$$

Creatine is a constant constituent of muscle-substance, the flesh of fowls being said to contain 0·32 per cent., cod-fish 0·17, and beef 0·07 per cent. Creatine has also been found in nerve-tissue, and probably occurs in minute traces in animal fluids. But its isolation from these does not prove its pre-existence, since it is very readily formed by the dehydration of creatinine, into which body, on the other hand, creatine is very easily changed.

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[1] Bocklisch isolated methyl-guanidine from impure cultures of *Vibrio proteus* in beef-broth. The ptomaïnes were precipitated by mercuric chloride in the manner directed by Brieger (page 336), and the precipitate decomposed by sulphuretted hydrogen in the usual way. The filtrate from the mercuric sulphide was concentrated and treated with a solution of sodium picrate, which precipitated the methyl-guanidine together with creatinine and cadaverine. On boiling the precipitate with absolute alcohol, the cadaverine picrate remained undissolved. The filtrate was evaporated, and the residue taken up with water, the solution acidulated with hydrochloric acid and shaken with ether to remove the picric acid, and the aqueous layer treated with auric chloride, when the gold salt of methyl-guanidine was precipitated, that of creatinine remaining in solution.

Creatine is most conveniently prepared from Liebig's extract of meat,[1] which sometimes contains granular crystals of the base. The extract should be dissolved in about 20 parts of water and the solution precipitated by a slight excess of basic acetate of lead. The filtered liquid is treated with sulphuretted hydrogen, again filtered, and concentrated to a syrup at a moderate temperature avoiding ebullition. Creatine crystallises out on standing in a cool place for some days. A more complete precipitation is effected if 2 or 3 volumes of alcohol be added. The precipitate of creatine is collected on a filter, washed with rectified spirit, and recrystallised from water.

Creatine is a white opaque substance, but it crystallises with one molecule of water in colourless, transparent rhombic prisms (fig. 13), which, when heated to 100° C., lose their water and become opaque. Creatine is soluble in 75 parts of cold water, and very soluble in hot. It is only very slightly soluble in absolute alcohol, more soluble in dilute spirit, and insoluble in ether.

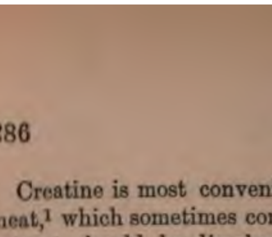
Fig. 13.—CREATINE (after Frey).

On cooling a strong aqueous solution of creatine, the base separates in bulky needles. On more gradual evaporation of a dilute solution it is deposited in large prisms.

The aqueous solution of creatine has a slightly bitter taste, and is neutral to litmus. With acids, creatine reacts as a monacid base, and combines to form crystallisable salts. The sulphate forms slender prisms, and the nitrate and hydrochloride thick short prisms. Creatine also unites with various neutral salts to form crystallisable compounds. That with zinc chloride forms small crystals, decomposed by water into its constituents.

Conversion of creatine into creatinine, with loss of the elements of water, takes place when solutions of creatine salts are heated above 30° C. Also, on passing a current of hydrochloric acid gas over solid creatine, it is converted into creatinine hydrochloride. On evaporating a solution of creatine with the calculated quantity of dilute sulphuric acid, it yields creatinine sulphate. Conversion also occurs very readily on boiling creatine with dilute hydrochloric acid, and the resultant creatinine can be readily identified by conversion into the zinc chloride compound. Creatine is also ompletely converted into creatinine by heating for ten hours ith dilute acetic acid.

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[1] Creatine was first isolated by Chevreul, in 1835, from a commercial meat extract or bouillon on which he was requested to report.

When boiled with baryta-water, creatine is decomposed into urea nd sarcosine, methyl-hydantoin being also formed. Sarcosine nd urea are also formed when creatine is heated in a sealed ube to 150° with an alkaline solution of barium chloride. The rea is further decomposed by the treatment, so that $2NH_3+CO_2$ re formed from one molecule of creatine. By heating with hosphoric acid to 150°, creatine yields methyl-hydantoin and ne molecule of ammonia, whereas urea yields $2NH_3$ under similar reatment. On heating an aqueous solution of creatine with ercuric oxide, oxalic acid and methyl-guanidine are ormed :—$C_4H_9N_3O_2+2HgO=C_2H_2O_4+C_2H_7N_3+2Hg$.

If to 2 c.c. of a cold saturated solution of creatine, five or six rops of a 20 per cent. solution of silver nitrate be added, and then ufficient solution of caustic potash to dissolve the precipitate rst formed, the liquid soon sets to a transparent jelly, and on eating separation of metallic silver takes place.

Creatine does not precipitate a solution of zinc chloride, a ehaviour which distinguishes it from creatinine.

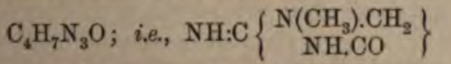
Creatine is also distinguished from creatinine by being unpre-ipitated by a solution of phospho-tungstic acid in presence of ydrochloric acid.

Creatine reduces Fehling's copper solution on long boiling, but o separation of cuprous oxide takes place.

When heated with nitrous acid, one-half the nitrogen of crea-ine is evolved in the free state. With alkaline hypobromite, wo-thirds of the nitrogen is said to be evolved. When heated ith soda-lime, creatine yields methylamine.

Kobert (*Chem. Zeit.*, 1888, p. 1662) recommends creatine as valuable excitant of muscular action, in the case of the heart, igestive organs, and general muscular system.

Creatinine. Methyl-glycocyamidine.



$$C_4H_7N_3O;\ \textit{i.e.},\ NH{:}C\left\{\begin{matrix}N(CH_3).CH_2\\ NH.CO\end{matrix}\right\}$$

Creatinine is an anhydride of creatine, $C_4H_9N_3O_2$ (page 285), nd is produced from the latter body with great facility. Creatin-ne occurs constantly in normal human urine, the amount varying, ccording to Voit, from 0·5 to 4·9 grammes per diem, according o the quantity of proteids eaten.[1] The proportion is not diminished by fasting, but is said to be increased in typhus, intermittent fever, pneumonia and tetanus, and diminished in convalescence from acute diseases, anæmia, chlorosis, paralysis and phthisis. Creatinine has been found in sweat and in the muscles of fishes, and G. S. Johnson has isolated creatinine, or a modification of it, from the flesh of a healthy cow.

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[1] According to J. L. W. Thudichum, the nitrogen eliminated by the idneys in the form of bases other than urea is normally about 11 per cent.

Creatinine may be isolated from human urine by Liebig's process, which consists in exactly neutralising the liquid with milk of lime, and adding calcium chloride as long as calcium phosphate continues to be precipitated. The filtrate, which should be neutral or very faintly acid, is evaporated to a small bulk, and the crystals of common salt, &c., removed. Thirty-two parts of the mother-liquor are treated with one part of zinc chloride in very concentrated solution, and the whole left for several days. The creatinine-zinc chloride, which separates in nodules, is washed with a little cold water, and then with alcohol. It is then boiled with recently precipitated lead hydroxide, the filtrate evaporated, and the residue digested with absolute alcohol, which dissolves the creatinine, leaving any creatine insoluble.[1]

Heintz and Pettenkofer neutralise fresh urine with sodium carbonate, evaporate to a syrup, exhaust the syrup with alcohol, and add alcoholic zinc chloride to the solution.

G. Stillingfleet Johnson (*Proc. Royal Soc.*, 1888, xliii. 507) has devised a method of isolating urinary creatinine, based on the formation of a compound of creatinine with mercuric chloride as originally proposed by Maly.[2] Johnson treats a large volume of the urine with 5 per cent. of its volume of a saturated aqueous solution of sodium acetate, and 25 per cent. of a saturated solution of mercuric chloride. The precipitate which forms contains all the uric acid, xanthine, and phosphates of the uri

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of the total nitrogen, some 4 per cent. being excreted in the form of creatin
Thudichum found the output of creatinine and creatine together to aver
¾ gramme per diem.

E. Ackermann (abst. *Jour. Chem. Soc.*, 1896, ii. 121) finds that a m
on mixed diet, and doing regular work, excretes, on the average, 1·25 gram
of creatinine daily.

[1] G. S. Johnson regards the creatinine obtained by Liebig's process having undergone some change. It is more soluble in alcohol than the b
obtained by his process (page 288), in which heating is wholly avoided, a
it forms an anhydrous chloroplatinate.

[2] Maly (*Ann. Chem. Pharm.*, clix. 279) recommends a preliminary con
tration of the urine by heat, and precipitation by basic acetate of lead bef
adding mercuric chloride. Johnson's modification is a marked improveme
on this, as it gives a purer product and wholly avoids the use of heat, wh
is an essential condition if it be desired to obtain unchanged creatinine.

ith any albuminous matters which may be present. The is filtered off immediately, and the filtrate left at rest ght hours. The creatinine gradually separates in micro- rical masses of the mercuric chloride compound. This off, washed with cold water, and decomposed by sul- hydrogen. The filtered liquid is decolorised with imal charcoal, and concentrated by spontaneous evapora- ulphuric acid, when creatinine hydrochloride separates h-yellow prisms. The crystals are redissolved in 15 ld water, and the cold solution treated with an excess droxide, freshly prepared by precipitating a solution of with ammonia. The mixture is well stirred for twenty nd then filtered. The filtrate is free from lead and and on evaporation over sulphuric acid in a vacuum creatinine in the form of efflorescent crystals containing $+2H_2O$. If this creatinine be dissolved in cold water, olution allowed to evaporate *in vacuo* at the ordinary re, the hydrated crystals are reobtained; but if it be in boiling water the crystals obtained on spontaneous n are anhydrous tables, which, according to G. S. Johnson, emically identical with the efflorescent form, but recon- to it by modifying the process of solution. The follow- hows the results obtained by Johnson by the recrystal- "artificial" urinary creatinine (*Proc. Royal Soc.*, xliii. oduced from creatine which had been prepared by boiling eatinine with water :—

Crystals.	Solvent.	Evaporation.	Product.
Kα, . . .	Water at 60° C.	In vacuo.	Tabular Kβ.
atinine α,	Water at 60° C.	In vacuo.	Efflorescent Kβ.
Kα, . . .	Water at 100° C.	In vacuo.	Tabular Kα (anhydrous).
β (anhy-	Cold water.	In vacuo.	Efflorescent Kα.
Kβ, . .	Cold water.	In vacuo.	Efflorescent Kβ.

Johnson has also employed the mercury process for the of creatinine from flesh (*Proc. Royal Soc.*, 1891, l. 287), ase obtained therefrom appears to be an isomer of urinary . Johnson gives the following characters showing the s between the creatinines obtained from the two sources mercury method and that regenerated from urinary

	Efflorescent Creatinine *a* of Urine. $C_4H_7N_3O.2H_2O$.	Tabular Creatinine *a* of Urine. $C_4H_7N_3O$.	Efflorescent Creatinine (Artificial) from Urinary Creatine. $C_4H_7N_3O.2H_2O$.	Tabular Creatinine (Artificial) from Urinary Creatine. $C_4H_7N_3O$.	Sarcous Creatinine.	Liebig's "Creatinine."
Solubility in water for original substance,	1 in 10·6 at 14° C.	1 in 10·78 at 17° C.	...	1 in 10·68 at 16·5° C.	1 in 10·74 at 13·7° C.	1 in 11·5 at 16° C.
Solubility in alcohol, .	...	1 in 362 at 17° C.	...	1 in 324 at 18·5° C.	1 in 490·2 at 13·7° C.	1 in 102 at 16° C.
Platinum salt, . . .	Indefinite or decomposed by alcohol.	$B_2H_2PtCl_6+2H_2O$.	Indefinite or decomposed by alcohol.	$B_2H_2PtCl_6+2H_2O$.	$B_2H_2PtCl_6+2H_2O$.	$B_2H_2PtCl_6$.
Solubility of platinum salt in water,	...	1 in 14·1 at 15° C.	...	1 in 24·4 at 15° C.	1 in 22·6 at 15° C.	...
Gold salt, ...	Unchanged by ether.	Unchanged by ether.	Decomposed by ether.	Decomposed by ether.	Soluble in ether, decomposed on evaporation.	...
Reduction of CuO compared with that of glucose,	$4C_4H_7N_3O = 2C_6H_{12}O_6$.	$4C_4H_7N_3O = 2C_6H_{12}O_6$.	$5C_4H_7N_3O = 2C_6H_{12}O_6$.	$5C_4H_7N_3O = 2C_6H_{12}O_6$.	$9C_4H_7N_3O = 4C_6H_{12}O_6$.	$6C_4H_7N_3O = 2C_6H_{12}O_6$.

sides the differences appearing in the table, Johnson states sarcous creatinine appears in the efflorescent form only after lution has been kept at 60° C. for some time, whereas the al creatinine of urine, when prepared most carefully without is always efflorescent ($C_4H_7N_3O,2H_2O$).

hnson is of opinion that sarcous creatinine is probably really nt in the fresh muscle-substance; but having regard to the mely slow separation of its mercury salt (many months being red for its complete precipitation), he thinks it just possible it may result from gradual changes effected in some closely- substance by the prolonged action of solution of mercuric ide.

the varieties of creatinine the characters of which are shown in bove table, J. L. W. Thudichum (*Med. Press*, 1895, p. 567) another, obtained from human urine by precipitation with pho-molybdic acid, with subsequent conversion into the zinc ide compound and the gold salt. This modification had no ing action on Fehling's solution.

view of Johnson's and Thudichum's results, and of the ence of the series of bases, closely allied to creatinine, isolated autier from flesh by treat- with absolute alcohol (page , it seems probable that al closely-allied substances been confused and described r the name of "creatinine."

ordinarily obtained from , creatinine crystallises in ne rhombic prisms and stellate (fig. 14). It dissolves in 11 parts of cold water, s sparingly soluble in alcohol, nsoluble in ether.

ie aqueous solution of creati- is, according to some observers, al, but according to others ine.[1] The solution readily undergoes change with formation eatine, especially if ammonia, oxide of lead, or other base esent. By prolonged boiling with caustic alkali, creatinine is letely decomposed.

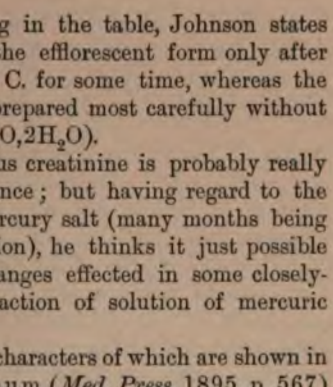
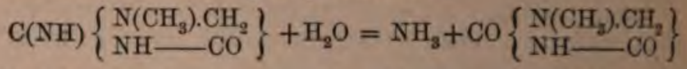


Fig. 14.—Creatinine (after Frey).

strongly alkaline sample leaves an alkaline ash on ignition, proving esence of mineral impurity. Salkowski finds creatinine quite free alkaline reaction, but it liberates ammonia from ammoniacal salts on g.

By boiling with baryta-water, creatinine is hydrolysed to ammonia and methyl-hydantoin:—



$$C(NH)\left\{\begin{matrix} N(CH_3).CH_2 \\ NH\text{——}CO \end{matrix}\right\} + H_2O = NH_3 + CO\left\{\begin{matrix} N(CH_3).CH_2 \\ NH\text{——}CO \end{matrix}\right\}$$

Boiled with water and mercuric oxide, it gives methyl-guanidine and oxalic acid (compare creatine). Heated with an alkaline solution of barium chloride, under pressure to 150°, creatinine behaves like creatine, but is only partially decomposed by phosphoric anhydride at the same temperature.

Creatinine yields a series of crystallisable salts. The *hydrochloride*, B,HCl, crystallises in short transparent prisms from alcohol or in large laminæ from water. It unites with zinc chloride to form the double salt $ZnCl_2,2BHCl$. This is very soluble in water and alcohol, and must not be mistaken for the compound $ZnCl_2,2C_4H_7N_3O$, which is one of the most characteristic salts of creatinine. *Creatinine-zinc chloride* is obtained by mixing concentrated aqueous or alcoholic solutions of zinc chloride and creatinine, or by adding sodium acetate to the solution of the double hydrochloride. It forms oblique rhombic prisms or small needles, which have a tendency to form rosettes or warty concretions. The crystals are soluble in about 54 parts of cold or 27 of boiling water. They are insoluble in absolute alcohol, and require 9217 parts of alcohol of 98 per cent., or 5734 of alcohol of 87 per cent. for their solution.

Mercuric chloride gives a white, curdy precipitate in strong solutions of creatinine, but the separation is not perfect unless sodium acetate be added, or mercuric acetate substituted for mercuric chloride. On allowing such a mixture to stand at the ordinary temperature, the compound is gradually deposited in microscopic spherules.[1] This reaction is applied by G. S. Johnson to the isolation of creatinine from urine (*Proc. Royal Soc.*, 1888, xliii. 507). The compound is almost insoluble in cold water, and is decomposed with partial reduction of the mercury by hot water. It is readily soluble in dilute hydrochloric acid, but is nearly insoluble in acetic acid. Johnson attributes to the spherical mercury compound the formula $C_{16}H_{28}N_{12}Hg_7Cl_{10}$, and suggests the following constitution :—

$$4(C_4H_5Hg''N_3O,HCl),3HgCl_2+2H_2O.$$

---

[1] The tendency of the compound to aggregate into spherules exists even after drying. Specimens in the author's laboratory, on inspection twelve months after being prepared, have been found to have altered their condition of apparent powder to that of a collection of spherical granules, some of which approach the size of a pin's head.

Experiments on specimens of the spherical salt prepared in the author's laboratory, both from urine and from pure creatinine prepared by Johnson's process, do not fully confirm this formula. The creatinine ranges from 16 to nearly 20 per cent., and the proportion of chlorine varies greatly in different preparations, a fact which points to the presence of HgO, and possibly of Hg_2Cl_2, in some specimens.

From a concentrated solution of creatinine, silver nitrate precipitates crystals of the compound, $C_4H_7N_3O,AgNO_3$. Mercuric nitrate does not precipitate a dilute solution of creatinine till excess of sodium carbonate is added, when $B_2,Hg(NO_3)_2,HgO$ is thrown down as a crystalline precipitate.

Creatinine possesses marked reducing properties. The mercury of the spherical salt above described is at once reduced, even in the cold, to the mercurous state and partly to metal on adding caustic alkali; contact with boiling water produces a similar change.

Creatinine reduces Fehling's solution on boiling, the blue liquid changing to yellow, but no cuprous oxide separates.[1] Creatinine appears also to prevent the separation of a precipitate when glucose is present, and hence exerts an interfering action on the application of Fehling's solution to the detection of dextrose in urine (Allen's *Chemistry of Urine*, p. 65). Pavy's solution is reduced by creatinine without precipitation, and may be used for its determination. According to G. S. Johnson, the reducing power of creatinine obtained direct from urine is greater than that of the base prepared from creatine (compare page 289). Thus he finds 12 grammes of tabular creatinine α from urine to have a cupric oxide reducing power equivalent to 10 grammes of glucose, that is, two molecules of this creatinine equal one of glucose in reducing power, against $2\frac{1}{2}$ molecules required of creatinine prepared from creatine.

Phospho-molybdic and phospho-tungstic acids produce microcrystalline precipitates in solutions of creatinine acidulated with nitric or hydrochloric acid. By treating the precipitates with baryta, free creatinine is obtained.

If a concentrated solution of picric acid be added to normal human urine a small crystalline sediment is gradually formed. On separating this, and treating it with hot water, uric acid remains, while the greater part dissolves. The soluble portion is a double picrate of potassium and creatinine, which forms lemon-yellow needles or thin prisms, readily soluble in hot water, sparingly in cold alcohol, and almost insoluble in ether. With dog's urine the precipitate produced by picric acid contains little or no uric acid, and the kynurenic acid present is not precipitated.

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[1] See C. Giacomo (*Chem. Centr.*, 1884, p. 185; abst. *Jour. Chem. Soc.*, xlviii. 702). O. Maschke (*Zeits. anal. Chem.*, 1878, p. 134; abst. *Jour. Chem. Soc.*, xxxv. 688) has described a white compound of creatinine with cuprous oxide obtained by adding Fehling's solution and glucose to a dilute solution of creatinine.

When a solution of picric acid is added to a solution of creatinine not more dilute than 1 in 3000, on adding a drop of dilute caustic alkali a blood-red colour is produced, which is intensified by boiling the liquid. By this reaction the presence of creatinine can be recognised in the urine of man, dog, and rabbit. Acetone gives a similar but less intense colour. Glucose gives a similar reaction on heating. It is evident that the behaviour of creatinine with picric acid gravely affects the value of that reagent as a test for small quantities of sugar in urine.

T. Weyl (*Berichte*, 1878, page 228) has pointed out that if a few drops of very dilute solution of sodium nitroprusside be added to a solution of creatinine, and dilute caustic soda then added drop by drop, a fine ruby-red colour will be produced, which in a few minutes changes to an intense straw-yellow. If the liquid be now acidulated with acetic acid and warmed, it turns greenish and prussian blue separates. Guareschi recommends that 10 per cent. solutions of nitroprusside and caustic soda should be used. Krugenberg states that the reaction is best obtained by first adding caustic soda, and then a few drops of a concentrated solution of the nitroprusside. Salkowski confirms this. The reaction is very delicate, and can be obtained with a solution containing 0·03 per cent. of pure creatinine, or with urine containing 0·066 per cent. In applying the test to urine the absence of acetone should be insured by distilling off a portion, since that body gives a ruby-red colour with Weyl's test, though no blue colour can be obtained on acidulating, acetic acid merely restoring the yellow colour to red.[1] According to Guareschi, a red colour is also yielded by hydantoin, methyl-hydantoin, and other compounds containing the group $N.CH_2.CO.N$. Creatine gives no reaction with Weyl's test unless the liquid be first boiled with a dilute acid, so as to convert it into creatinine. In this manner, Weyl demonstrated the presence of creatine in milk (*Berichte*, xi. 2175).

The determination of creatinine in urine is usually based on its lation as creatinine-zinc chloride, which process is preferred by eubauer. E. Salkowski directs that 240 c.c. of the urine ould be rendered alkaline by the cautious addition of milk of ne, and precipitated by calcium chloride. The volume is made to 300 c.c., and the liquid filtered after ten minutes. 250 c.c. the filtrate, representing 200 of urine, which must be feebly kaline, is evaporated to about 20 c.c., and an equal measure of solute alcohol added. This is subsequently diluted to 100 c.c. th alcohol, allowed to stand twenty-four hours, and filtered. 80 c.c. of the filtrate, slightly acidified with acetic acid, zinc loride is added, and the precipitate collected after twenty-four urs. The purity of the creatinine-zinc chloride should be oved by a microscopic examination, with a high power, to make rtain of its freedom from sodium chloride. It should be com- etely soluble in hot water (compare page 292).

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[1] G. Colasanti (abst. *Jour. Chem. Soc.*, 1887, p. 1056) states that Weyl's reaction for creatinine is given by urine which has undergone the ammoniacal fermentation for some time, as also by urine which has been preserved for many months in sterilised vessels.

Instead of weighing the compound of creatinine with zinc loride, the contained creatinine may be deduced from the ount of ammonia produced on decomposing it with boiling ncentrated sulphuric acid. For this purpose the precipitate ould be dissolved in the minimum quantity of sulphuric acid, eviously diluted with an equal measure of water, and the solu- n treated as in Kjeldahl's process.

P. Grocco (*Chem. Centr.*, 1887, page 17) has described a dification of the zinc process.

A method of isolating creatinine from urine which, in the inion of the author, is preferable to the zinc process, is that sed on its precipitation as the spherical mercuric compound scribed on page 288. Instead of weighing the mercury salt, e composition of which is variable, it should be decomposed by ong sulphuric acid, as in Kjeldahl's process, the creatinine being duced from the amount of ammonia obtained, or the measure of trogen evolved on treatment with hypobromite.

A limited number of experiments, made in the author's boratory by A. R. Tankard, appear to show that fairly good timations of creatinine may be made by treating the mercury zinc compound with soda and bromine, as described on page 273. ne nitrogen evolved is 50 per cent. of the total, a result difficult explain if the accepted formula for creatinine be correct.

Gautier's Flesh Bases.

A. Gautier (*Jour. de Pharm.*, [5], xiii. 354; *Jour. Chem.* c., l. 634) has described a series of bases, closely allied to crea- nine, which he isolated from the flesh of large animals by eatment with absolute alcohol. These bases are definite crystalline bodies, which when administered to animals act more or less powerfully on the nerve-centres, inducing sleep, and in some cases causing vomiting and purging, in a manner similar to the alkaloids of snake-poison, but less powerfully than the ptomaïnes. These bases are formed during life (leucomaïnes) and occur in the urine, saliva, venom, and various glandular secretions, but Gautier has chiefly studied their occurrence in muscle.

XANTHOCREATININE, $C_5H_{10}N_4O$, is the most abundant of the bases isolated by Gautier from muscle by treatment with absolute alcohol. It closely resembles creatinine, from which it differs by CH_3N. Xanthocreatinine forms sulphur-yellow spangles having a slightly bitter taste. On warming, it evolves an odour resembling acetamide. It is very soluble in water, and also dissolves in boiling absolute alcohol. When taken internally it causes sleep, diarrhœa, and vomiting. It turns blue litmus paper red, but appreciably changes red litmus to blue. Xanthocreatinine forms a hydrochloride crystallising in feathery needles. It also yields crystalline compounds with mercuric and zinc chlorides. The platinum salt is very soluble, and crystallises in long sheaves. The gold salt crystallises with difficulty. Xanthocreatinine gives a precipitate with phospho-molybdic acid, and with silver nitrate a flocculent precipitate soluble in hot water, and crystallising therefrom in needles. Xanthocreatinine is not precipitated by nitric or oxalic acid, nor by cupric acetate; this last reaction distinguishing it from the bases of the xanthine-group.

Xanthocreatinine has been detected in human urine, especially after fatigue.

CHRYSOCREATININE OR CRUSOCREATININE, $C_5H_8N_4O$, resembles creatinine, from which it differs by CHN. It forms beautiful orange-yellow crystals, sparingly soluble in water. The reaction of the solution is strongly alkaline. The base forms a non-deliquescent hydrochloride and a soluble crystalline chloroplatinate. The aurichloride forms slightly soluble crystalline grains. Crusocreatinine is not precipitated by nitric or oxalic acid, or by cupric acetate even on boiling. It precipitates alumina from a solution of alum.

AMPHICREATININE, $C_9H_{19}N_7O_4$, only exists in small quantity in muscle. It is a feeble base, crystallising in pale yellow prisms, which, on heating to 100°, become opaque, but do not change their form. It has a bitter taste, and is only slightly soluble in cold water. It forms a crystalline non-deliquescent hydrochloride, a platinum salt crystallising in lozenge-shaped plates, soluble in water but insoluble in alcohol. The gold salt forms hexahedra and tetrahedra, very soluble in water. Amphicreatinine is not precipitated by mercuric chloride or cupric acetate, and gives no reaction with the murexide test.

Pseudoxanthine, $C_4H_5N_5O$, closely resembles xanthine. It is described on page 315.

Two Unnamed Bases, containing $C_{11}H_{24}N_{10}O_5$ and $C_{12}H_{25}N_{11}O_5$ respectively, are described by Gautier as occurring, together with the foregoing, in muscular tissue.

The following table exhibits in a condensed form the method employed by Gautier for the extraction of the bases above described. It is evident from the process employed that their oxalates are soluble in absolute alcohol.

Fresh meat, finely minced, is digested with tepid water containing in each litre 0·25 gramme of oxalic acid, and 2 c.c. hydrogen peroxide. At the end of twenty-four hours the liquid is strained off, the last portions being recovered by pressure, the liquid boiled to coagulate albumin, and again filtered, when the filtrate is treated in the following manner:—

The liquid is evaporated to dryness at 50° C., preferably under reduced pressure, and the residue extracted with cold absolute alcohol. The alcoholic solution is evaporated to dryness and then extracted with hot absolute alcohol, the liquid decanted and allowed to stand. Ether (spec. grav., 0·725) is added to complete precipitation. The solution is allowed to stand, when after a time (varying with the purity of the ether used) a mass of crystals separates out, which is filtered off and washed with cold absolute alcohol.

<table>
<tr><td rowspan="3">lcoholic Washings. porate to dryness, e up residue with ter, add slight excess cupric acetate, and ng to the boiling nt. Filter from pre- ltate, suspend it in water, and pass phuretted hydrogen ough the hot liquid, l filter hot. The fil- te on cooling deposits <i>udoxanthine</i>.</td><td colspan="4">B. Crystals are treated with boiling absolute alcohol, and the liquid filtered hot.</td></tr>
<tr><td colspan="2">Solution is allowed to become cold and filtered.</td><td colspan="2">Residual crystals are dissolved in hot water, and the liquid allowed to cool, and filtered after standing.</td></tr>
<tr><td>Filtrate. Add to alcoholic washings at A.</td><td>Crystals consist of <i>xanthocreatinine</i> and two unnamed bases of the formula $C_{11}H_{24}N_{10}O_5$ and $C_{12}H_{25}N_{11}O_5$.</td><td>Filtrate. Concentrate by evaporation and allow to stand, when <i>crusocreatinine</i> separates out.</td><td>Crystals consist of— <i>amphicreatinine</i>.</td></tr>
</table>

J. L. W. Thudichum (*Comp. rend.*, cvi. 1803; *Med. Press*, 1895) has described certain bases from urine, some of which are possibly identical with Gautier's flesh bases. Thudichum states that normal urine contains six bases precipitable by phosphomolybdic acid, and that these represent about 11 per cent. of the total nitrogen excreted in the urine. He specifies them as *creatinine*, $C_4H_7N_3O$; *urotheobromine*, $C_7H_8N_4O_2$, apparently identical with the base described by G. Salomon under the name of paraxanthine; *reducine*, $C_6H_4N_3O_4$;[1] *urochrome*, and two *bases* unnamed and unformulated. If the phospho-molybdates of the mixed bases be decomposed with baryta and barium carbonate, any excess of baryta got rid of by passing carbon dioxide, and the deep red liquid filtered, a solution of the free bases is obtained. If this be treated boiling hot with very dilute ferric chloride, Thudichum states that the urochrome is precipitated, and that, if the liquid be filtered hot, the filtrate deposits clouds of urotheobromine on cooling. The iron compound of urochrome is described as very soluble in dilute acids and in excess of alkali. It may be decomposed by sulphuretted hydrogen; or dissolved in dilute sulphuric acid, and reprecipitated by phospho-molybdic acid.[2]

SEPARATION OF FLESH CONSTITUENTS.—The following table, compiled from various sources, gives a systematic method for the separation of the leading constituents of flesh. It may also be applied to extract of meat, urine, and other animal products.

---

[1] Reducine forms a barium compound almost insoluble in alcohol. Neutral or acid solutions of the base reduce ferric, cupric, and mercuric salts to ferrous, cuprous, and mercurous salts respectively, and silver salts to metallic silver.

[2] J. L. W. Thudichum announced the discovery and isolation of urochrome in 1871. He has since described it at great length on several occasions, but has never published a formula for it. The whole of Thudichum's statements in this connection require verification, and meanwhile should be accepted with considerable reserve. The following description of urochrome is taken from the *Medical Press* for March 6th, 1895:—

Urochrome, isolated as described in the text, is stated to have the properties of a weak base and of a feeble acid, and to exhibit an amphibolic reaction with litmus. It contains at least 20·9 per cent. of nitrogen, but its exact composition has not been established. Urochrome is regarded by Thudichum as the chief if not the only colouring matter of normal urine. In the solid state, urochrome is said to form an amorphous yellow or red-brown brittle resin, having a strong urinous odour. Urochrome dissolves in water in all proportions to form an intensely orange-yellow liquid. It is also soluble in rectified spirit, less readily in absolute alcohol, and is insoluble in ammonia, ether, chloroform, or benzene. Urochrome is precipitated by adding ether to its alcoholic solution. On subsequently adding a trace of hydrochloric acid, the precipitate entirely dissolves, a behaviour attributed with improbability to the formation of a hydrochloride.

The spectrum of urochrome shows no definite absorption-bands. Urochrome exhibits no fluorescence with zinc chloride and ammonia. Its solution is decolorised by nascent hydrogen, and the colour is not restored by hydrogen peroxide. An aqueous solution becomes brown on standing. Ammonia pre-

<table>
<tr><td colspan="11">with cold water, repeating the extraction
mixed extracts, boil, and filter.</td></tr>
<tr><td rowspan="6">Precipitate consists of *albumin*.</td><td colspan="10">Filtrate. Add basic lead acetate, avoiding excess, allow to stand 48 hours, and filter.</td></tr>
<tr><td colspan="3" rowspan="3">Precipitate consists of compounds of lead with phosphoric and uric acids, and inosite.
Suspend precipitate in warm water, add a few drops of a solution of sodium carbonate, and pass sulphuretted hydrogen gas through the liquid, and filter. Boil the filtrate to expel sulphuretted hydrogen, and add lead acetate, avoiding excess, and filter.</td><td colspan="7">Filtrate. Pass sulphuretted hydrogen gas, filter, and make filtrate up to 250 c.c. Allow to stand several days, and filter from any amorphous or crystalline deposit. Wash the deposit with alcohol.</td></tr>
<tr><td rowspan="4">Deposit consists of *creatine*.</td><td colspan="6">Filtrate. Divide into two portions.</td></tr>
<tr><td colspan="3">I. Boil off alcohol, and dilute to 150 c.c., make slightly alkaline with ammonia, and add a solution of ammonio-nitrate of silver. Filter and wash precipitate, which consists of compounds of silver nitrate with xanthine, hypoxanthine, and guanine, with dilute ammonia. Dissolve the precipitate in boiling nitric acid, specific gravity 1·1, and decant liquid from any AgCl. Allow solution to stand for six hours, and filter from any crystals that may form.</td><td colspan="3">II. Evaporate to low bulk, and filter from any crystals.</td></tr>
<tr><td colspan="2">Precipitate. Decompose with sulphuretted hydrogen, and filter. Add HCl to filtrate, and allow to stand several days. Filter from any deposit.</td><td rowspan="2">Filtrate contains the inosite. Remove the excess of lead by means of sulphuretted hydrogen, and filter. Filtrate will contain any *inosite*.</td><td rowspan="2">Crystals. Treat with ammonio-nitrate of silver (to neutralise any adhering nitric acid), suspend in boiling water, and pass sulphuretted hydrogen gas through the liquid. Filter, and allow filtrate to stand, when crystals of *hypoxanthine* will form.</td><td colspan="2">Filtrate. Add slight excess of ammonia, and filter off the precipitated xanthine silver compound. Decompose precipitate, suspended in boiling water, with sulphuretted hydrogen gas, and filter hot. Concentrate filtrate, which on cooling will deposit xanthine and guanine. Treat crystals with dilute ammonia.</td><td colspan="2">Crystals consist of leucine, tyrosine, and perhaps creatine. The crystals are treated with hot 70 per cent. alcohol, and filtered.</td><td rowspan="2">Filtrate is treated with solutions of sodium acetate and syrupy zinc chloride, when *creatinine* will be precipitated.</td></tr>
<tr><td>Deposit consists of *uric acid*.</td><td>Filtrate contains the *phosphoric acid*.</td><td>Residue. Dissolve in HCl, and add cold saturated solution of picric acid, when *guanine* picrate will be precipitated.</td><td>Filtrate contains the *xanthine*.</td><td>Residue of crystals, insoluble in alcohol, consists of *tyrosine*, which may be purified by crystallisation from boiling water.</td><td>Filtrate. On cooling, *leucine* crystallises out, which may be purified as follows:—
Boil for half an hour with dilute potash containing a little oxide of lead. The lead sulphide is filtered off, the filtrate exactly neutralised with dilute sulphuric acid, and evaporated to dryness at 100° C. The residue is extracted with boiling alcohol to extract the *leucine*.</td></tr>
</table>

Indole. C_7H_7N; *i.e.*, $C_6H_4\left\{\begin{matrix}.CH:\\ .NH.\end{matrix}\right\}CH$; or $HN\left\{\begin{matrix}-C_6H_4\\ CH:CH\end{matrix}\right\}$

Indole is so named from being the nucleus from which the indigo-group of chemical compounds is derived. It has been obtained synthetically by several reactions, and is, together with skatole, a characteristic constituent of fæces. Its occurrence therein is due to the putrefactive decomposition of proteids which usually takes place to a greater or less extent in the intestinal canal. The greater part of the skatole and indole formed is eliminated by the kidneys in the forms of skatoxyl- and indoxyl-sulphuric acids (the so-called "urinary indican"), the remainder being excreted in the fæces.

Indole is formed when albuminous substances are fused with caustic potash, but it is more easily obtained by digesting liver or fibrin with water and putrid pancreas.[1] If the digestion be not too prolonged, the product when acidified and distilled yields a distillate from which impure indole can be extracted by agitation with ether. On evaporation, the ether leaves a residue of indole mixed with skatole and phenol. From the last body indole can be purified by dissolving it in benzene and adding a benzene solution of picric acid, when indole picrate, $C_8H_7N,C_6H_3(NO_2)_3O$,

serves urochrome, even in presence of caustic alkali, but free acid readily decomposes it. If boiled for a short time with water containing 5 per cent. of sulphuric acid, urochrome is decomposed with formation of a red precipitate. From this, when filtered off, washed and dried, ether is stated to extract omicholic acid and omicholin, while subsequent treatment with absolute alcohol dissolves uropittin, $C_9H_{10}N_2O_3$ (by many chemists called urobilin), leaving uromelanin, insoluble in water, but dissolved readily by very dilute soda, and reprecipitated by acid.

Urochrome is said to be entirely removed from its alkaline solutions by ag tation with animal charcoal, and to be completely precipitated by phosph molybdic and phospho-tungstic acids, and also by mercuric chloride. Wi benzoyl chloride and caustic soda it is stated to yield an immediate prec pitate of a benzoyl-derivative of indefinite composition, which may be purifi by boiling with water, and is soluble in spirit. The same substance m be obtained by dissolving the phospho-tungstate or phospho-molybdate urochrome in strong caustic soda and agitating the solution with benzoy chloride.

[1] Harris and Tooth (*Journ. Physiol.*, ix. 220) found that the form tion of indole by putrid pancreatic digestion was very capricious. A qua tity of mercuric chloride or phenol insufficient to render the fluid asept prevented the formation of indole. Whenever indole was present, howeve large numbers of various kinds of bacteria were also found; but bacteria we also abundant in the absence of indole. Hence it appears probable th certain indole-forming organisms exist.

es out in long red needles, which are sparingly soluble in
zene, readily in hot, very slightly soluble in petroleum-
d decomposed by ammonia.

skatole, indole may be separated by dissolving the mixture
mallest possible quantity of absolute alcohol, and then
om 8 to 10 volumes of water, when the skatole will be
ted while the indole remains in solution.

pure, indole forms crystalline scales of a satiny lustre.
persistent and disgusting fæcal odour, melts at 52°, and
h partial decomposition at about 245°. Indole distils
a current of steam. It is very soluble in alcohol, ether,
oleum-spirit, and dissolves with moderate facility in hot
parating on cooling in oily drops, which subsequently
tes resembling benzoic acid.

possesses feeble basic characters. When treated with
ydrochloric acid it forms a sparingly soluble salt which
posed by boiling with water. The picrate has already
cribed.

aqueous solution of indole be treated with fuming nitric
preferably with a solution of sodium nitrite acidified with
or nitric acid, a red precipitate is formed of the composition
$O)N_2,HNO_3$. A deep red coloration is produced when an
solution of indole is treated with nitrous acid, or nitrogen
passed through it, but the red needles which are deposited
d to have a different composition from the above.

strip of pine wood be moistened with strong hydrochloric
immersed in an alcoholic solution of indole, or exposed
pours of indole, it is coloured deep crimson.

a dilute solution of indole is treated with sodium nitro-
and a few drops of caustic soda solution added, a violet-
oration is produced, which changes to pure blue when the
acidulated with acetic acid.

elting a minute quantity of indole in a test-tube with
ted oxalic acid, a fine magenta coloration is produced.
uring matter formed is soluble in acetic acid.

is decomposed when boiled with moderately-concentrated
oda, which behaviour distinguishes it from skatole.

LE, or METHYL-INDOLE,[1] C_9H_9N, closely resembles indole.
r is somewhat similar and equally persistent and unplea-
t crystallises from hot water or, preferably, petroleum-
glittering white scales, melts at 95°, and boils at 265°.

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scher attributes to skatole the following constitutional formula :—

$$C_6H_4\left\{\begin{array}{l}.C(CH_3):\\ .NH.CH:\end{array}\right\}$$

Skatole gives no colour-reactions with pine wood and hydrochloric acid, nor with sodium nitroprusside, and also differs from indole in not suffering decomposition when boiled with moderately concentrated caustic soda. The picrate is precipitated in red needles on mixing hot aqueous solutions of skatole and picric acid. When sodium nitrite is added to a solution of skatole in glacial acetic acid, a dark brown coloration is produced, and on adding water the nitrosamine is precipitated as a yellow oil, which solidifies in a freezing mixture to a crystalline mass. E. Fischer describes the formation of this compound as highly characteristic, and available for the detection of skatole and its separation from indole.

A methyl-indole, which has been obtained by synthetical means, has an aromatic odour, and is liquid at ordinary temperatures.

INDOXYL, $C_8H_5(NH).OH$, is interesting as a body intermediate between indole and indigotin. It possesses both basic and acid characters. Its alkaline solution absorbs atmospheric oxygen with formation of indigo-blue, which is also produced on adding ferric chloride to a hydrochloric acid solution of indoxyl.

INDOXYL-SULPHURIC ACID, $C_8H_5(NH).SO_4H$,[1] occurs in urine as a potassium salt. The normal excretion contains only traces of this compound, but which received the unfortunate name of "urinary indican," from a supposed identity with plant-indican, t glucoside from which indigo is obtained. The only similari between the two bodies is that both yield indigo-blue as one the products of their decomposition.[2]

Potassium indoxyl-sulphate, $C_8H_5(NH).SO_4K$, crystallises fr hot alcohol in colourless lustrous tables, readily soluble in wat but only sparingly in cold alcohol. When boiled with dilute ac it is decomposed into indoxyl and acid potassium sulphate, but not attacked by alkalies. When the crystals are heated, ind gotin (indigo-blue) sublimes, and the same substance is form quantitatively when the acidulated solution is warmed with fer chloride.

For the detection of indoxyl-sulphuric acid in urine, Jaf (*Pflüger's Archiv.*, iii. 448) first separates any albumin by boili e liquid, and treats the filtrate with an equal measure of hydroloric acid. A dilute solution of bleaching powder is then utiously added, until the blue colour no longer increases. On itating with chloroform the colouring matter is taken up and can obtained on evaporation. Jaffe's method is not suitable for the etection of traces of indigogen, as the colouring matter is destroyed y the least excess of the oxidising agent. Hence MacMunn boils he urine with an equal measure of hydrochloric acid and a few rops of nitric acid, cools, and agitates with chloroform. The hloroform is generally coloured violet, and, when examined in the pectroscope, shows two broad absorption-bands, one on either side f the D line. The less refrangible is due to indigo-blue and the more refrangible to indigo-red; though it is doubtful if the latter olouring matter is identical with the indirubin which occurs in ommercial indigo.[1]

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[1] Indoxyl-sulphuric acid is described by some writers as indoxy sulphonic acid. The latter name would be applicable to a body of t constitution $C_8H_4(SO_3H)(NH).OH$. This would be isomeric with indoxy sulphuric acid, and would not exhibit the readiness of the latter in hydr lysing into sulphuric acid and indoxyl.

[2] Decomposing urine occasionally forms a bluish-red pellicle, and ultimat deposits microscopic crystals of indigo-blue. A calculus of the same natu has been described.

A. C. **Méhu** (*Jour. Pharm.*, [5], vii. 122) adds to the urine bout 0·5 c.c. of strong sulphuric acid to 1 litre of the sample, nd then saturates the liquid with powdered ammonium sulphate, hereby any indigotin or indirubin is precipitated.[2] On treating he precipitate in the cold with proof-spirit the indirubin will be issolved, while the insoluble indigotin is purified by washing ith water, followed by spontaneous drying. Méhu proposes a olorimetric process for the estimation of indigotin, which he issolves in hot carbolic acid to which sufficient glycerin or bsolute alcohol has been added to prevent crystallisation on ooling. The colour of a solution of indigo-blue of known rength, prepared in this manner, is compared with that of the rinary pigment.

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[1] For the detection of indirubin, O. **Rosenbach** (*Jour. Chem. Soc.*, lviii. 32) adds nitric acid to the boiling urine, cools, adds a large excess of mmonia, and agitates with ether, which will acquire a purple colour if indibin be present. For its isolation, Rosenbach treats the fresh urine with ad acetate, heats the filtered liquid to boiling, and adds nitric acid, drop y drop, until a purple colour is produced, carefully avoiding excess of acid. he liquid is then cooled and treated with ammonia till alkaline. The prepitate is filtered off, washed in succession with ammonia, dilute hydrochloric id, and water, and then dissolved in boiling alcohol. The solution deposits digo-blue on cooling. It is filtered and the filtrate treated with alcoholic ad acetate, again filtered, and most of the alcohol boiled off. On diluting e residual liquid with water, impure indirubin is precipitated as a brown owder, which, after washing with water, may be purified by crystallisation om chloroform or ether.

[2] Apparently, Méhu's method is intended to apply to ready-formed indiotin and indirubin, but in Michailoff's process it appears to be the indigogens hich are precipitated by ammonium sulphate.

W. Michailoff (*Jour. Chem. Soc.*, liv. 880) also saturates the acidified urine with finely powdered ammonium sulphate, and then extracts the urobilin by repeated agitations with ethyl acetate (acetic ether). The aqueous layer is next mixed with an equal volume of fuming hydrochloric acid, chloroform added, and then cautiously treated with dilute bromine-water, agitating well between each addition. By presenting the indigo with the solvent when in the nascent state its extraction is said to be very readily and perfectly effected.[1]

Indoxyl-sulphuric acid occurs in very small quantities in normal human urine, Jaffe finding from ·004 to 0·019 gramme in 1500 c.c. of the excretion. Horse's urine contains twenty-three times as much.[2] The proportion in human urine is much increased in certain diseases, such as cholera, typhus, peritonitis, dysentery, and Addison's disease. In obstructive diseases of the small intestine the increase is enormous. The presence of a large amount of indigogens in the urine generally implies that abundant albuminous putrefaction is in progress in some part of the system, these putrefactive products being absorbed and eliminated by the kidneys in the forms of indoxyl-sulphuric acid and its analogue skatoxyl-sulphuric acid, $C_8H_6(CH_3)N.SO_4H$. The latter body is said to be somewhat more abundant in human urine than the indoxyl-compound. When decomposed by hydrochloric acid or an oxidising agent, it gives a colouring matter usually reddish, but which may possess a marked purple tint.

Traces of compounds of indoxyl and skatoxyl with glycuronic acid not improbably exist in normal urine, and their proportions appear to be greatly increased under certain conditions.

XANTHINE BASES. ALLOXUR BASES.

Xanthine is the typical member of a series of feebly basic bodies closely related to uric acid and to each other. Some of these compounds occur in small quantity in urine and animal tissues, and are normal products of the degradation of proteids. Others members of the group, *e.g.*, caffeine, theobromine, theophylline, and xanthine itself, occur in plants. The following tabular arrangement of the chief members of the group shows their composition and relation to each other and to uric acid.

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[1] All the oxidising agents mentioned in the text are liable to destroy the indigo-blue if used in excess. A preferable plan is to employ ferric chloride in presence of hydrochloric acid.

[2] From 25 litres of normal dog's urine, J. Hoppe-Seyler (*Jour. Chem. Soc.*, xlvi. 1058) isolated 1 gramme of crystallised potassium indoxyl-sulphate and 0·5 gramme of potassium phenyl-sulphate. Neither orthocinnamic acid, orthoamidocinnamic acid, or orthonitrobenzaldehyde, alone or with acetone, produced any increase in the quantity of indigogens excreted.

Sarkine or Hypoxanthine $\}$ $C_5H_4N_4O$.	Xanthine, $C_5H_4N_4O_2$	Uric acid, $C_5H_4N_4O_3$
.. ..	Methylxanthine Heteroxanthine $\}$ $C_6H_6N_4O_2$	.. ..
.. ..	Dimethylxanthines :— Paraxanthine Theobromine Theophylline $\}$ $C_7H_8N_4O_2$	Carnine, $C_7H_8N_4O_3$
.. ..	Trimethylxanthine :— Caffeine, $C_8H_{10}N_4O_2$	.. ..
Imidosarkine :— Adenine, $C_5H_5N_5$	Imidoxanthine :— Guanine, $C_5H_5N_5O$	.. ..
.. ..	Pseudoxanthine, $C_4H_5N_5O$	.. ..

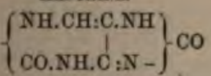
From this table it appears that hypoxanthine, xanthine, and uric acid differ from each other by an atom of oxygen. Notwithstanding this close relationship, they do not seem to be convertible. The alleged reduction of uric acid to xanthine and hypoxanthine, by treatment with sodium amalgam, and the oxidation of hypoxanthine to xanthine by means of nitric acid, were not authentic.[1]

The vegetable bases, *theobromine*, *theophylline*, and *caffeine*, are

---

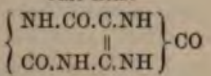
[1] E. Fischer (*Ber.*, xxviii. 3135) has recently shown that the difference between uric acid and xanthine is not merely in the number of oxygen atoms, but also in the situation of the double linkage and of the hydrogen atoms, as indicated by the following formulæ :—

XANTHINE.



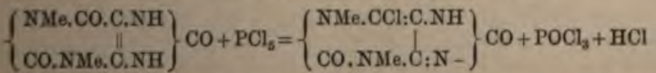
$$\left\{\begin{array}{r} \mathrm{NH.CH{:}C.NH} \\ | \\ \mathrm{CO.NH.C{:}N-} \end{array}\right\}\mathrm{CO}$$

URIC ACID.



$$\left\{\begin{array}{r} \mathrm{NH.CO.C.NH} \\ \| \\ \mathrm{CO.NH.C.NH} \end{array}\right\}\mathrm{CO}$$

In the methyl-derivatives of uric acid previously known, the structure of the carbon-chain has remained unaltered, while the hydrogen and oxygen have been removed from the alloxan-nucleus. Recently Fischer and Ach succeeded in replacing both hydrogen atoms of the alloxan-nucleus by methyl, and thus obtained γ-dimethyluric acid, convertible by phosphorus pentachloride into the chloro-derivative of theophylline, thus :—



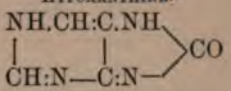
$$\left\{\begin{array}{r} \mathrm{NMe.CO.C.NH} \\ \| \\ \mathrm{CO.NMe.C.NH} \end{array}\right\}\mathrm{CO}+\mathrm{PCl_5}=\left\{\begin{array}{r} \mathrm{NMe.CCl{:}C.NH} \\ | \\ \mathrm{CO.NMe.C{:}N-} \end{array}\right\}\mathrm{CO}+\mathrm{POCl_3}+\mathrm{HCl}$$

By reduction with hydriodic acid the chlor-theophylline is converted into theophylline, and this by methylation yields caffeine.

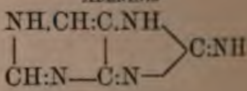
methylated xanthines. *Guanine* is an imidoxanthine, while *adenine* bears the same relationship to hypoxanthine.

The constitution of the alloxur-bases is not known in every case, but the following formulæ rest on a sufficiently sure foundation :—

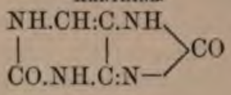
HYPOXANTHINE.[1]



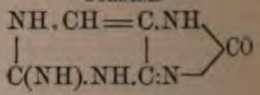
ADENINE.[1]



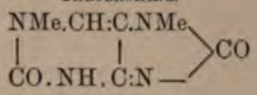
XANTHINE.



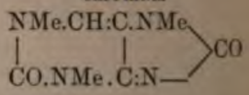
GUANINE.



THEOBROMINE.



CAFFEINE.



In their chemical and physical characters, the xanthine bases present a close resemblance to uric acid. They have but a feeble affinity for acids, and their salts are mostly decomposed by water. Some of them (including xanthine itself) exercise an acid function in addition, and unite with bases. They are mostly very slightly soluble in cold water, and, except caffeine and theobromine, are insoluble in alcohol, ether, or chloroform. They all yield white precipitates with phospho-molybdic acid, mercuric chloride, and ammoniacal lead acetate ; and guanine and adenine are very perfectly precipitated by picric acid.

A general reaction of the xanthine bases (including uric acid) is their precipitation from ammoniacal solutions by ammonio-nitrate of silver, as a gelatinous compound of the base with argentic oxide. The xanthine compound contains $C_5H_4N_4O_2Ag_2O$. The precipitates are usually insoluble in ammonia, unless concentrated and used in large excess, but to ensure complete precipitation excess should be avoided. On treating the precipitates with dilute nitric acid of 1·10 specific gravity, they are converted into compounds of the bases with silver nitrate, xanthine forming

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[1] The formulæ given for hypoxanthine and adenine are those assigned them by Krüger in his latest researches (*Zeit. physiol. Chem.*, xviii. 423, 459 ; abst. *Jour. Chem. Soc.*, 1894, i. 212). He assigns to the imido-group eliminated by nitrous acid a different position from that shown in the formula for guanine.

$C_5H_4N_4O_2,AgNO_3$. These compounds are well-defined crystallisable bodies, insoluble in water, and, in the cases of hypoxanthine, carnine, adenine and episarkine, insoluble in nitric acid of the above strength, even on boiling; or, at any rate, crystallising out rapidly on cooling. Guanine, carnine, adenine, and episarkine are stated by G. Salomon (*Zeits. physiol. Chem.*, xviii. 207) to behave similarly, but, according to J. L. W. Thudichum, the silver nitrate compound of guanine dissolves tolerably easily in hot dilute nitric acid, and is only very gradually deposited on cooling.[1] The compounds of xanthine, heteroxanthine, and paraxanthine remain in solution after cooling, which difference of behaviour permits of their separation from the bases previously mentioned. The bases are all completely reprecipitated as their silver-oxide compounds on neutralising the nitric acid solution by ammonia. Heteroxanthine and paraxanthine may be separated from xanthine by taking advantage of the limited solubility of their sodium salts in caustic soda, and from each other by utilising the sparing solubility of the hydrochloride of heteroxanthine.

Most of the xanthine bases are precipitated by cupric acetate, especially on heating. A still more perfect separation is effected by cuprous solutions, the precipitate consisting in each case of the cuprous salt of the derivative. These compounds may be obtained by treating the neutral solution with a mixture of cupric sulphate and sodium sulphite or thiosulphate, or by mixing the ammoniacal solution with Fehling's solution, heating to boiling, and gradually adding a solution of dextrose. Instead of cuprous oxide separating in the free state, it combines with the xanthine derivative to form a white insoluble compound. Hence it is evident that the presence of xanthine and its allies, including uric acid, will prevent the detection of sugar in urine by Fehling's test to an extent dependent on the amount of the interfering body present. The fact is of considerable practical importance when small quantities of sugar are to be sought for.

If, instead of using dextrose, the mixture of the alloxur-base with Fehling's solution be treated with hydroxylamine hydrochloride, reduction of the copper to the cuprous state occurs in the strongly alkaline solution and at the ordinary temperature. Treated in this way, *guanine* and *xanthine* give precipitates which are at first white, but rapidly become green by oxidation. *Heteroxanthine* and *paraxanthine* give similar white precipitates. The *uric acid* precipitate, cuprous urate, $Cu_2O,C_5H_4N_4O_3$, is yellowish-white at first, but rapidly becomes greenish. The *carnine* precipitate is liable to be mixed with uncombined cuprous oxide, which colours it yellow. The *adenine* and *hypoxanthine* precipitates are white. *Theobromine* and *caffeine* are the only known xanthine derivatives which do not give precipitates with the above reagent.

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[1] G. Bruhns (*Berichte*, xxiii. 225) utilises the same behaviour for the separation of xanthine and guanine from hypoxanthine and adenine.

P. Balke (*Jour. prakt. Chem.*, [2], xlvii. 537) employs the foregoing reaction for the determination of the xanthine derivatives. For this purpose the substance is dissolved in alkali to a solution of not less than 1 per cent. in strength, a few c.c. of a solution of hydroxylamine hydrochloride added, and then Fehling's solution run in gradually, the liquid being constantly agitated until the yellowish-white precipitate first formed commences to turn yellowish-red, indicating that the xanthine derivatives are entirely precipitated, and cuprous oxide is being thrown down. The reaction is better observed if the precipitate be allowed to settle a little, and a short interval should be allowed to elapse between each addition of the Fehling's solution, as the reaction is not very rapid. In all cases, Cu_2 added as Fehling's solution corresponds to one molecule of xanthine bases precipitated. The results are always somewhat below the truth.

M. Krüger (*Zeits. physiol. Chem.*, xviii. 351) finds that, by the employment of cupric sulphate and sodium hydrogen sulphite, all the xanthinoid bodies containing a substituted NH-group in the molecule are precipitated very perfectly from warm solutions as cuprous compounds. Theobromine constitutes a remarkable exception to this rule, being, like caffeine, creatine and creatinine, unaffected by Krüger's reagent. In carrying out the method, Krüger slightly acidulates the liquid containing the xanthine bases with sulphuric acid. Sodium bisulphite is then added, and this is followed by cupric sulphate, when the precipitable bases are thrown down as gelatinous or flocculent white precipitates, which gradually become green or brown. In some cases the solution must be heated to ensure complete precipitation, but in others the reaction occurs perfectly in the cold. The precipitates dissolve readily in mineral acids, but only with difficulty in hot acetic acid. They are not altered by caustic soda, but dissolve in ammonia in presence of air. They are readily decomposed by alkaline sulphides.

Uric acid is completely precipitated by Krüger's reagent, as also are adenine, methyl-adenine, hypoxanthine, and guanine. Dimethyl-hypoxanthine is not precipitated from warm solutions, but from cold concentrated solutions it separates in fine yellow needles.

If sodium thiosulphate (hyposulphite) be substituted for sodium bisulphite, the behaviour of the xanthinoid bodies is somewhat different, apparently owing to the formation of compounds soluble in excess of the thiosulphate. Adenine is completely thrown down in cold solutions on standing, and methyl-adenine and guanine behave similarly, but hypoxanthine is not precipitated even from moderately strong solutions unless warmed. The following table shows the general behaviour of the xanthinoid bodies with Krüger's reagents :—

	Cupric Sulphate, and	
	Sodium Hydrogen Sulphite.	Sodium Thiosulphate.
Uric acid,	Precipitated.	Precipitated.
Adenine,	Ppted.	Ppted.
Methyl-adenine,	Ppted.	Ppted.
Hypoxanthine,	Ppted.	Ppted. on warming only.
Guanine,	Ppted.	Ppted.
Dimethyl-hypoxanthine,	Ppted. only from cold concentrated solutions.	Not ppted.
Theobromine,	Not ppted.	Not ppted.
Caffeine,	Not ppted.	Ppted.
Creatine,	Not ppted.	Not ppted.
Creatinine,	Not ppted.	Not ppted.

Krüger and **Wolff** (*Zeit. physiol. Chem.*) employ the following process for the determination of xanthine and the allied bases in urine. 100 c.c. of the sample, free from albumin, is heated to boiling and treated with 10 c.c. of a saturated solution of sodium bisulphite, followed immediately by 10 c.c. of a 13 per cent. solution of cupric sulphate, and the liquid again boiled. Five c.c. of a 10 per cent. solution of barium chloride is then added to promote settlement, and after two hours the precipitate is filtered off, and washed thoroughly with air-free water at 50° C. The precipitate, together with the filter, is then subjected to Kjeldahl's nitrogen process, and the excess of nitrogen present over that previously found to exist as uric acid is regarded as existing in the form of xanthine and its analogues.

E. **Salkowski** (abst. *Jour. Chem. Soc.*, 1895, ii. 538) precipitates 1000 c.c. of urine by ammonio-nitrate of silver, after removing the phosphates by magnesia-mixture. The silver precipitate is suspended in water, decomposed by sulphuretted hydrogen, and the filtered liquid evaporated to dryness. The residue is treated with a little water containing from 2 to 3 per cent. of sulphuric acid, which dissolves the xanthine bases, leaving the uric acid practically insoluble. After twenty-four hours, the liquid is filtered and the xanthine bases reprecipitated by ammonio-nitrate of silver, and estimated from the weight of silver in the precipitate. By this process, Salkowski found the amount of xanthine bases to be about 8 to 10 per cent. of the uric acid. He states that they are not true xanthine but resemble hypoxanthine (compare pages 296, 297).

The proportion of xanthine derivatives (other than uric acid) ordinarily present in *urine* is extremely small, but there is reason to believe that, under circumstances not fully understood, their amount is much increased and may then be of pathological importance. For the actual isolation and separation of the xanthine bases, 5 to 10 gallons of urine should be treated by instalments of about 1 quart at a time with neutral lead acetate in powder, as long as a precipitate is produced. The liquid is filtered and sodium sulphate added as long as lead sulphate is thrown down, the liquid poured off from the precipitate, sodium bisulphite and copper sulphate added, and the liquid boiled. The precipitate, which contains the xanthine derivatives as cuprous salts, is filtered off, washed, dissolved in dilute nitric acid, and excess of ammonia added, followed by silver nitrate. The precipitate, consisting of the argentic oxide compounds of xanthine, &c., is separated, suspended in hot ammoniacal water, and decomposed by sulphuretted hydrogen, the resultant silver sulphide filtered off, and the filtrate concentrated till the xanthine crystallises out. If, instead of decomposing the silver precipitate with sulphuretted hydrogen, it be boiled with nitric acid of 1·10 specific gravity, the silver nitrate compounds of hypoxanthine and adenine will crystallise out immediately on cooling, while those of xanthine, paraxanthine, and heteroxanthine will remain in solution, and may be recovered as the silver oxide compounds by rendering the filtrate ammoniacal.

Balke (*Jour. prakt. Chem.*, [2], xlvii. 537) employs the [illegible] process for the isolation of the xanthine [illegible] gives the following details of an experiment:— [illegible] of finely-minced horse-flesh, from whi[illegible] viously removed as far as possibl[illegible] weight of water at 50° to 60° [illegible] agitation. The liquid was [illegible] similarly treated with half [illegible] obtained were boiled and [illegible]

[illegible] rendered alkaline by caustic soda, and [illegible] of phosphate. The filtrate was [illegible] and Fehling's solution [illegible]. The supernatant liquid was decanted from [illegible] precipitate, which was washed by de- [illegible] and then collected on a [illegible]. The precipitate was next suspended in [illegible] by sulphuretted hydrogen. The liquid [illegible] concentrated, made [illegible] with lead acetate, by which the lead com- [illegible] hypoxanthine, and carnine were completely [illegible] which gave no trace of a pre- [illegible]. On boiling the lead precipitate [illegible] the carnine compound was dissolved, [illegible] from the solution after treating it [illegible] hydrogen. The weight isolated was [illegible]. The portion of the lead precipitate insoluble in boiling [illegible] sulphuretted hydrogen, when, by evapor- [illegible] a mixture of xanthine and hypoxanthine was [illegible] mass weighing 0·172 gramme. This [illegible] nitrate, and the bases separated [illegible] with nitric acid of 1·10 specific gravity. [illegible] of bodies of the xanthine-group from malt, [illegible] kilogrammes of the dry grain with water con- [illegible] per cent. of sulphuric acid. The liquid is strained, [illegible] to half its bulk, and at once precipitated with neutral [illegible] in excess. The filtered liquid is treated with sul- [illegible] hydrogen, filtered, again heated to dissipate the excess

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[illegible] half of the liquid was treated by Neubauer's process as follows:— [illegible] filtrate was treated with lead acetate, filtered, and the excess of [illegible] by sulphuretted hydrogen. The filtrate was evaporated to a [illegible], and allowed to stand for several days. The creatine which [illegible] was filtered off with the aid of a mercury-pump, and washed with [illegible] spirit. The filtrate and washings were heated on the water-bath, [illegible] added, and precipitated with ammonio-nitrate of silver. The pre- [illegible] was separated, [illegible] with dilute ammonia, and boiled with dilute [illegible] (speci[illegible] 1·10) containing a little urea. The liquid was [illegible] id of a hot-water funnel, and allowed to stand [illegible] hours, [illegible] xanthine compound crystallised out in small [illegible] which, [illegible] nd converted into the hypoxanthine silver com- [illegible] a. From the acid mother-liquor xanthine [illegible] adding ammonia. From the silver com- [illegible] hine the bases were obtained as yellowish- [illegible] g sulphuretted hydrogen and evaporating of the precipitant, and treated at 80° C. with a solution of cupric sulphate. The precipitate, which contains the xanthine bases, is separated, decomposed by sulphuretted hydrogen, and the filtrate again treated with cupric sulphate. After again decomposing the precipitate with sulphuretted hydrogen, the filtered and concentrated liquid is neutralised with ammonia and precipitated with silver nitrate, the precipitated xanthine bases being separated as before.

Xanthine. $C_5H_4N_4O_2$.

Xanthine differs from hypoxanthine and from uric acid by an atom of oxygen. Its constitutional formula is given on page 306. Xanthine was originally discovered by Marcet (1819) in a urinary calculus, and called "xanthic oxide." It has been found in Jarvis Island guano, occurs as a normal constituent of urine especially during the use of sulphur-baths, and is present in minute quantities in yeast, the muscles of mammals and fishes, the liver, spleen, pancreas, thymus, brain, &c. It occurs also in very small quantities in plants, *e.g.*, in tea, malt-seedlings, lupins, &c. The only natural source from which it can be at all conveniently extracted is meat-extract. It has been produced synthetically by A. Gautier (*Jour. Chem. Soc.*, xlviii. 275) by the reaction of hydrocyanic acid and water in presence of acetic acid, but its best mode of preparation is the decomposition of guanine by nitrous acid:— $C_5H_5N_5O + HNO_2 = C_5H_4N_4O_2 + H_2O + N_2$. A nitro-compound is formed at the same time, which yields xanthine on reduction.[1]

Pure xanthine forms a white powder consisting of microscopic crystals. It acquires a waxy lustre by friction. On heating xanthine, a small portion sublimes unchanged, but by far the greater part chars, with evolution of cyanogen, hydrocyanic acid, carbon dioxide, and ammonia.

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[1] A boiling solution of guanine in nitric acid is treated with sodium nitrite until red nitrous fumes are copiously evolved, when the liquid is largely diluted with water. The resultant yellow precipitate is washed and dissolved in hot ammonia, and a solution of ferrous sulphate added until black ferroso-ferric hydrate is precipitated. The liquid is then filtered, evaporated to dryness, and the residue washed with water to dissolve out ammonium sulphate. It is then dissolved in ammonia and the solution allowed to evaporate.

According to E. Fischer (*Annalen*, ccxv. 253) a preferable plan is to dissolve 10 grammes of guanine in a mixture of 20 grammes of concentrated sulphuric acid with 150 c.c. of water. After boiling, the liquid is cooled to about 80°, and 8 grammes of sodium nitrite added with constant agitation. The yield of xanthine is nearly quantitative, the product only of a pale orange colour, and free from the above-mentioned nitro-compound.

ine is very sparingly soluble in water, requiring 1400 boiling or 14,000 of cold water for solution. The hot solution deposits a pellicle on evaporation. Its reaction l. In alcohol and ether xanthine is insoluble.

ine dissolves in dilute acids and in alkalies, and unites h class of bodies to form crystallisable compounds (fig. 15). ves with facility in caustic potash or soda, but is precipi- m the solution by adding an acid, even carbonic acid. It ed by warm ammonia (distinction from uric acid), and on rystals of the ammonium salt separate; but on exposure on evaporating the solution, all the ammonia is lost and thine remains.

$H_3N_4O_2+H_2O$ crystallises in microscopic needles from a of xanthine in the smallest possible quantity of caustic ike acid urate of sodium, it is decomposed by repeated lisation, and retains its water of crystallisation till heated 190° C. On boiling xanthine with baryta-water, the soluble barium salt separates on cooling.

ine hydrochloride, B,HCl, is deposited in difficultly soluble g scales aggregated in nodules. The *sulphate*, B,H_2SO_4 forms microscopic glistening tables, which lose the whole acid on washing er. *Xanthine ni-* rms fine yellow of characteristic pic appearance (fig.

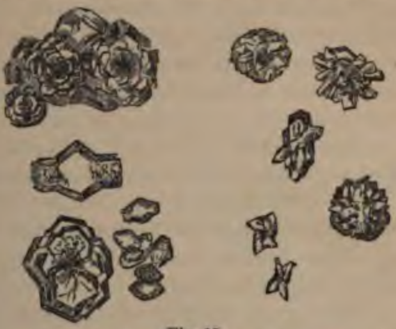

Fig. 15.

XANTHINE NITRATE. XANTHINE HYDROCHLORIDE.

(After Kühne.)

ric chloride preci- anthine from very lutions. A solu- part of xanthine 0 gives a distinct ice with mercuric

acetate produces ipitate in a cold of xanthine, but on heating a flocculent precipitate of en colour is formed. With cuprous salts, or with solution in presence of hydroxylamine hydrochloride 7), the compound $Cu_2O,C_5H_4N_4O_2$ is thrown down as a ecipitate, which rapidly turns green from oxidation.

moniacal solution of xanthine gives precipitates with the of calcium and zinc, and with lead acetate.

moniacal solution of xanthine gives a gelatinous precipitate of $Ag_2O,C_5H_4N_4O_2$ with ammonio-nitrate of silver. Treated with hot dilute nitric acid (sp. gr., 1·10) it dissolves, and the solution, after cooling, very slowly (if at all) deposits crystals of xanthine-silver nitrate, $C_5H_4N_4O_2,AgNO_3$, grouped in a manner resembling wavellite. The same compound separates when a solution of xanthine in a minimum of nitric acid is treated with silver nitrate. Its greater solubility in hot nitric acid of the above strength distinguishes the silver nitrate compound of xanthine from those of hypoxanthine, carnine, adenine (and guanine); while those of (guanine,) hypoxanthine and paraxanthine resemble the xanthine compound in their behaviour.

Xanthine gives the following colour-reactions with oxidising agents:—

Strecker's Test.—Xanthine dissolves in hot nitric acid without evolution of gas. On careful evaporation of the solution a yellow residue remains, which turns reddish-yellow on addition of caustic potash or soda, and on subsequent heating becomes reddish-violet. If ammonia be substituted for the fixed alkali in the above test, no violet coloration is obtained. This behaviour distinguishes xanthine from uric acid, which gives the characteristic murexide reaction when similarly treated.

Weidel's Test.—If xanthine be treated with freshly-prepared chlorine-water and a trace of nitric acid, and the liquid carefully evaporated to dryness, a residue is obtained which becomes pink or crimson on cautious exposure to ammoniacal vapours (compare uric acid).

Hoppe-Seyler's Test.—If solid xanthine be sprinkled on a sol[illegible] tion of caustic soda with which some bleaching powder has be[illegible] mixed, each particle becomes surrounded with a dark green ri[illegible] or scum, which rapidly becomes brown and disappears.

When treated with hydrochloric acid and potassium chlora[illegible] xanthine yields **alloxan** and **urea**.

Xanthine occurs in very rare cases as a urinary calculus. F[illegible] its detection, the powdered calculus should be boiled with caust[illegible] alkali, and the filtered solution treated with hydrochloric acid a[illegible] again filtered. If xanthine be present in any quantity, hexagon[illegible] tables or globular masses of the hydrochloride will form as t[illegible] liquid cools. The indication may be confirmed by dissolving t[illegible] product in ammonia and adding ammonio-nitrate of silver, wh[illegible] gelatinous xanthine-silver oxide will be precipitated.

Flesh, meat-extract, and similar matters may be examined f[illegible] xanthine by the method described in the table on page 29[illegible] From urine, xanthine may be isolated by the processes detailed [illegible] page 309 *et seq.*

PSEUDOXANTHINE, $C_4H_5N_5O$, is a base discovered by Gautier in iscular tissues. It is described as forming a yellow powder, or llate crystals, much resembling uric acid. It is sparingly soluble water, but soluble in alkalies. It forms a very soluble hydro-oride, and yields precipitates with ammoniacal lead acetate, rcuric chloride, and ammonio-nitrate of silver. With oxidising nts it gives similar colour-reactions to xanthine.

HETEROXANTHINE, $C_6H_6N_4O_2$. This base, which has probably constitution of a methyl-xanthine,[1] occurs in very small antity in normal human urine, together with xanthine, paraxan-ne and hypoxanthine. It is said to be present in larger amount the urine of anæmic patients. Heteroxanthine has also been ected in dog's urine. It is crystalline, soluble with difficulty cold water, but much more readily on heating, and is insoluble alcohol or ether. The *hydrochloride* crystallises readily and is y sparingly soluble, which fact gives a means of separating eroxanthine from the closely-allied base paraxanthine, the drochloride of which is more easily soluble. The two bases y be separated from co-occurring xanthine derivatives by the ring solubility of their sodium salts in excess of caustic soda.

Heteroxanthine gives an insoluble compound with ammonio-rate of silver. It yields a brilliant colour with Weidel's test ge 314), but gives no characteristic reaction with Strecker's test.

PARAXANTHINE, $C_7H_8N_4O_2$, has probably the constitution of a nethyl-xanthine, and is isomeric with theobromine and theophyl-e (Part ii. page 498). It exists in minute quantity in urine. raxanthine is sparingly soluble in cold water, but dissolves readily warming, and is insoluble in alcohol and ether. It crystallises flat, irregular, hexagonal tables when its solution is slowly porated, but if the liquid be rapidly concentrated the base arates in needles. Paraxanthine is said to be poisonous.

The silver nitrate compound of paraxanthine is soluble in hot ute nitric acid, thus resembling the xanthine compound and ferentiating paraxanthine from hypoxanthine, carnine, adenine, sarkine, and guanine. Paraxanthine responds to Weidel's re-ion, but gives no colour with Strecker's test.

Paraxanthine is further distinguished from xanthine by its ater solubility in water, and from heteroxanthine by the more dy solubility of its hydrochloride (see above).

Guanine. Imido-xanthine. $C_5H_5N_5O$. (See page 306.) Guanine is best prepared from Peruvian guano, which should be is treated with a little water containing from 2 to 3 per cent. of sulphuric acid, which dissolves the xanthine bases, leaving the uric acid practically insoluble. After twenty-four hours, the liquid is filtered and the xanthine bases reprecipitated by ammonio-nitrate of silver, and estimated from the weight of silver in the precipitate. By this process, Salkowski found the amount of xanthine bases to be about 8 to 10 per cent. of the uric acid. He states that they are not true xanthine but resemble hypoxanthine (compare pages 296, 297).

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[1] A mono-methylxanthine has recently been found in the urine after taking ch tea and coffee.

The proportion of xanthine derivatives (other than uric acid) ordinarily present in *urine* is extremely small, but there is reason to believe that, under circumstances not fully understood, their amount is much increased and may then be of pathological importance. For the actual isolation and separation of the xanthine bases, 5 to 10 gallons of urine should be treated by instalments of about 1 quart at a time with neutral lead acetate in powder, as long as a precipitate is produced. The liquid is filtered and sodium sulphate added as long as lead sulphate is thrown down, the liquid poured off from the precipitate, sodium bisulphite and copper sulphate added, and the liquid boiled. The precipitate, which contains the xanthine derivatives as cuprous salts, is filtered off, washed, dissolved in dilute nitric acid, and excess of ammonia added, followed by silver nitrate. The precipitate, consisting of the argentic oxide compounds of xanthine, &c., is separated, suspended in hot ammoniacal water, and decomposed by sulphuretted hydrogen, the resultant silver sulphide filtered off, and the filtrate concentrated till the xanthine crystallises out. If, instead of decomposing the silver precipitate with sulphuretted hydrogen, it be boiled with nitric acid of 1·10 specific gravity, the silver nitrate compounds of hypoxanthine and adenine will crystallise out immediately on cooling, while those of xanthine, paraxanthine, and heteroxanthine will remain in solution, and may be recovered as the silver oxide compounds by rendering the filtrate ammoniacal.

Balke (*Jour. prakt. Chem.*, [2], xlvii. 537) employs the copper process for the isolation of the xanthine bases from flesh, and gives the following details of an experiment:—About 800 grammes of finely-minced horse-flesh, from which the nerves had been previously removed as far as possible, was digested with an equal weight of water at 50° to 60° C. for about an hour with constant agitation. The liquid was pressed through a cloth, and the residue similarly treated with half the quantity of water. The liquors obtained were boiled and filtered from the coagulated albumin.

One-half of the filtrate[1] was rendered alkaline by caustic soda, and filtered from a small precipitate of phosphates. The filtrate was treated with hydroxylamine hydrochloride, and Fehling's solution then gradually added. The supernatant liquid was decanted from the bulky yellowish-brown precipitate, which was washed by decantation with sodium acetate solution, and then collected on a filter and again washed. The precipitate was next suspended in ammonia, and decomposed by sulphuretted hydrogen. The liquid was filtered from the copper sulphide, concentrated, made ammoniacal, and treated with lead acetate, by which the lead compounds of xanthine, hypoxanthine, and carnine were completely precipitated, and a filtrate obtained which gave no trace of a precipitate with silver nitrate. On boiling the lead precipitate several times with water the carnine compound was dissolved, and the base was obtained from the solution after treating it with sulphuretted hydrogen. The weight isolated was 0·0697 gramme. The portion of the lead precipitate insoluble in boiling water was decomposed by sulphuretted hydrogen, when, by evaporating the filtrate, a mixture of xanthine and hypoxanthine was obtained, as a yellowish-white mass weighing 0·172 gramme. This was treated with ammonio-silver nitrate, and the bases separated by boiling the precipitate with nitric acid of 1·10 specific gravity.

For the isolation of bodies of the xanthine-group from *malt*, Balke boils several kilogrammes of the dry grain with water containing 0·5 per cent. of sulphuric acid. The liquid is strained, concentrated to half its bulk, and at once precipitated with neutral lead acetate in excess. The filtered liquid is treated with sulphuretted hydrogen, filtered, again heated to dissipate the excess of the precipitant, and treated at 80° C. with a solution of cupric sulphate. The precipitate, which contains the xanthine bases, is separated, decomposed by sulphuretted hydrogen, and the filtrate again treated with cupric sulphate. After again decomposing the precipitate with sulphuretted hydrogen, the filtered and concentrated liquid is neutralised with ammonia and precipitated with silver nitrate, the precipitated xanthine bases being separated as before.

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[1] The other half of the liquid was treated by Neubauer's process as follows:—The clear filtrate was treated with lead acetate, filtered, and the excess of lead removed by sulphuretted hydrogen. The filtrate was evaporated to a syrup at 100°, and allowed to stand for several days. The creatine which separated was filtered off with the aid of a mercury-pump, and washed with rectified spirit. The filtrate and washings were heated on the water-bath, ammonia added, and precipitated with ammonio-nitrate of silver. The precipitate was separated, washed with dilute ammonia, and boiled with dilute nitric acid (specific gravity 1·10) containing a little urea. The liquid was filtered boiling hot, with the aid of a hot-water funnel, and allowed to stand for four hours, when the hypoxanthine compound crystallised out in small needles which were filtered off and converted into the hypoxanthine silver compound by digestion with ammonia. From the acid mother-liquor xanthine silver oxide was precipitated by adding ammonia. From the silver compounds of xanthine and hypoxanthine the bases were obtained as yellowish-white crystalline masses by passing sulphuretted hydrogen and evaporating the filtrates.

Xanthine. $C_5H_4N_4O_2$.

Xanthine differs from hypoxanthine and from uric acid by an atom of oxygen. Its constitutional formula is given on page 30[illegible]. Xanthine was originally discovered by Marcet (1819) in a urinary calculus, and called "xanthic oxide." It has been found in Jarvis Island guano, occurs as a normal constituent of urine especially during the use of sulphur-baths, and is present in minute quantities in yeast, the muscles of mammals and fishes, the liver, spleen, pancreas, thymus, brain, &c. It occurs also in very small quantities in plants, *e.g.*, in tea, malt-seedlings, lupins, &c. The only natural source from which it can be at all conveniently extracted is meat-extract. It has been produced synthetically by A. Gautier (*Jour. Chem. Soc.*, xlviii. 275) by the reaction of hydrocyanic acid and water in presence of acetic acid, but its best mode of preparation is the decomposition of guanine by nitrous acid:— $C_5H_5N_5O + HNO_2 = C_5H_4N_4O_2 + H_2O + N_2$. A nitro-compound is formed at the same time, which yields xanthine on reduction.[1]

Pure xanthine forms a white powder consisting of microscopic crystals. It acquires a waxy lustre by friction. On heating xanthine, a small portion sublimes unchanged, but by far the greater part chars, with evolution of cyanogen, hydrocyanic acid, carbon dioxide, and ammonia.

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[1] A boiling solution of guanine in nitric acid is treated with sodium nitrite until red nitrous fumes are copiously evolved, when the liquid is largely diluted with water. The resultant yellow precipitate is washed and dissolved in hot ammonia, and a solution of ferrous sulphate added until black ferroso-ferric hydrate is precipitated. The liquid is then filtered, evaporated to dryness, and the residue washed with water to dissolve out ammonium sulphate. It is then dissolved in ammonia and the solution allowed to evaporate.

According to E. Fischer (*Annalen*, ccxv. 253) a preferable plan is to dissolve 10 grammes of guanine in a mixture of 20 grammes of concentrated sulphuric acid with 150 c.c. of water. After boiling, the liquid is cooled to about 80°, and 8 grammes of sodium nitrite added with constant agitation. The yield of xanthine is nearly quantitative, the product only of a pale orange colour, and free from the above-mentioned nitro-compound.

anthine is very sparingly soluble in water, requiring 1400 s of boiling or 14,000 of cold water for solution. The hot eous solution deposits a pellicle on evaporation. Its reaction eutral. In alcohol and ether xanthine is insoluble.

anthine dissolves in dilute acids and in alkalies, and unites h each class of bodies to form crystallisable compounds (fig. 15). issolves with facility in caustic potash or soda, but is precipi- d from the solution by adding an acid, even carbonic acid. It issolved by warm ammonia (distinction from uric acid), and on ling crystals of the ammonium salt separate; but on exposure ir, or on evaporating the solution, all the ammonia is lost and xanthine remains.

$NaC_5H_3N_4O_2+H_2O$ crystallises in microscopic needles from a tion of xanthine in the smallest possible quantity of caustic a. Like acid urate of sodium, it is decomposed by repeated ystallisation, and retains its water of crystallisation till heated about 190° C. On boiling xanthine with baryta-water, the ringly soluble barium salt separates on cooling.

Xanthine hydrochloride, B,HCl, is deposited in difficultly soluble tening scales aggregated in nodules. The *sulphate*, B,H_2SO_4 H_2O, forms microscopic glistening tables, which lose the whole their acid on washing h water. *Xanthine ni-te* forms fine yellow stals of characteristic roscopic appearance (fig.).

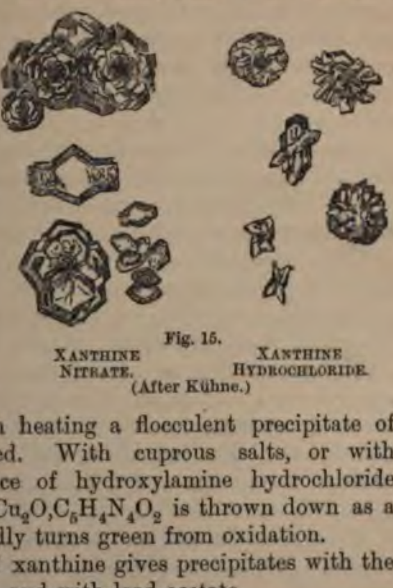

Fig. 15.

XANTHINE NITRATE. XANTHINE HYDROCHLORIDE.

(After Kühne.)

Mercuric chloride preci- tes xanthine from very te solutions. A solu- of 1 part of xanthine 0,000 gives a distinct escence with mercuric ride.

upric acetate produces precipitate in a cold tion of xanthine, but on heating a flocculent precipitate of e-green colour is formed. With cuprous salts, or with ing's solution in presence of hydroxylamine hydrochloride 307), the compound $Cu_2O,C_5H_4N_4O_2$ is thrown down as a e precipitate, which rapidly turns green from oxidation.

n ammoniacal solution of xanthine gives precipitates with the rides of calcium and zinc, and with lead acetate.

n ammoniacal solution of xanthine gives a gelatinous precipitate of $Ag_2O,C_5H_4N_4O_2$ with ammonio-nitrate of silver. Treated with hot dilute nitric acid (sp. gr., 1·10) it dissolves, and the solution, after cooling, very slowly (if at all) deposits crystals of xanthine-silver nitrate, $C_5H_4N_4O_2,AgNO_3$, grouped in a manner resembling wavellite. The same compound separates when a solution of xanthine in a minimum of nitric acid is treated with silver nitrate. Its greater solubility in hot nitric acid of the above strength distinguishes the silver nitrate compound of xanthine from those of hypoxanthine, carnine, adenine (and guanine); while those of (guanine,) hypoxanthine and paraxanthine resemble the xanthine compound in their behaviour.

Xanthine gives the following colour-reactions with oxidising agents:—

Strecker's Test.—Xanthine dissolves in hot nitric acid without evolution of gas. On careful evaporation of the solution a yellow residue remains, which turns reddish-yellow on addition of caustic potash or soda, and on subsequent heating becomes reddish-violet. If ammonia be substituted for the fixed alkali in the above test, no violet coloration is obtained. This behaviour distinguishes xanthine from uric acid, which gives the characteristic murexide reaction when similarly treated.

Weidel's Test.—If xanthine be treated with freshly-prepared chlorine-water and a trace of nitric acid, and the liquid carefully evaporated to dryness, a residue is obtained which becomes pink or crimson on cautious exposure to ammoniacal vapours (compare uric acid).

Hoppe-Seyler's Test.—If solid xanthine be sprinkled on a so tion of caustic soda with which some bleaching powder has be mixed, each particle becomes surrounded with a dark green ri or scum, which rapidly becomes brown and disappears.

When treated with hydrochloric acid and potassium chlora xanthine yields **alloxan** and **urea**.

Xanthine occurs in very rare cases as a urinary calculus. F its detection, the powdered calculus should be boiled with caus alkali, and the filtered solution treated with hydrochloric acid a again filtered. If xanthine be present in any quantity, hexago tables or globular masses of the hydrochloride will form as t liquid cools. The indication may be confirmed by dissolving t product in ammonia and adding ammonio-nitrate of silver, wh gelatinous xanthine-silver oxide will be precipitated.

Flesh, meat-extract, and similar matters may be examined xanthine by the method described in the table on page 2 From urine, xanthine may be isolated by the processes detailed page 309 *et seq.*

Pseudoxanthine, $C_4H_5N_5O$, is a base discovered by Gautier in muscular tissues. It is described as forming a yellow powder, or stellate crystals, much resembling uric acid. It is sparingly soluble in water, but soluble in alkalies. It forms a very soluble hydrochloride, and yields precipitates with ammoniacal lead acetate, mercuric chloride, and ammonio-nitrate of silver. With oxidising agents it gives similar colour-reactions to xanthine.

Heteroxanthine, $C_6H_6N_4O_2$. This base, which has probably the constitution of a methyl-xanthine,[1] occurs in very small quantity in normal human urine, together with xanthine, paraxanthine and hypoxanthine. It is said to be present in larger amount in the urine of anæmic patients. Heteroxanthine has also been detected in dog's urine. It is crystalline, soluble with difficulty in cold water, but much more readily on heating, and is insoluble in alcohol or ether. The *hydrochloride* crystallises readily and is only sparingly soluble, which fact gives a means of separating heteroxanthine from the closely-allied base paraxanthine, the hydrochloride of which is more easily soluble. The two bases may be separated from co-occurring xanthine derivatives by the sparing solubility of their sodium salts in excess of caustic soda.

Heteroxanthine gives an insoluble compound with ammonio-nitrate of silver. It yields a brilliant colour with Weidel's test (page 314), but gives no characteristic reaction with Strecker's test.

Paraxanthine, $C_7H_8N_4O_2$, has probably the constitution of a dimethyl-xanthine, and is isomeric with theobromine and theophylline (Part ii. page 498). It exists in minute quantity in urine. Paraxanthine is sparingly soluble in cold water, but dissolves readily on warming, and is insoluble in alcohol and ether. It crystallises in flat, irregular, hexagonal tables when its solution is slowly evaporated, but if the liquid be rapidly concentrated the base separates in needles. Paraxanthine is said to be poisonous.

The silver nitrate compound of paraxanthine is soluble in hot dilute nitric acid, thus resembling the xanthine compound and differentiating paraxanthine from hypoxanthine, carnine, adenine, episarkine, and guanine. Paraxanthine responds to Weidel's reaction, but gives no colour with Strecker's test.

Paraxanthine is further distinguished from xanthine by its greater solubility in water, and from heteroxanthine by the more ready solubility of its hydrochloride (see above).

Guanine. Imido-xanthine. $C_5H_5N_5O$. (See page 306.)

Guanine is best prepared from Peruvian guano, which should be boiled with milk of lime till it assumes a greenish-yellow colour, when the liquid is filtered. The operation is repeated as long as a coloured filtrate is obtained. The residue, which contains the whole of the uric acid and guanine, is repeatedly extracted with sodium carbonate. The filtrate is treated with sodium acetate, hydrochloric acid added to strong acid reaction, and the guanine dissolved out of the precipitate by boiling it with dilute hydrochloric acid. The guanine hydrochloride which separates on cooling is separated from admixed uric acid by boiling it with dilute ammonia, and the residual guanine dissolved in hot concentrated nitric acid, which on cooling deposits the nitrate, from which the free base may be liberated by ammonia. C. Wolff (*Zeit. Physiol. Chem.*, xvii. 468) recommends the following method for the preparation of guanine :—Guano is boiled for four hours with dilute sulphuric acid, cooled and filtered, the filtrate made alkaline with caustic soda and again filtered. To the filtrate ammoniacal nitrate of silver is added, which precipitates the guanine and uric acid. The washed precipitate is treated with hot dilute hydrochloric acid, the silver chloride filtered off, and the filtrate decolorised with animal charcoal. From the clarified liquid the guanine is precipitated by ammonia. It is redissolved in hot nitric acid containing a small quantity of urea (to ensure the absence of any trace of nitrous acid), and the liquid set aside to crystallise. The guanine nitrate which separates is free from uric acid, and is freed from traces of xanthine by solution in dilute caustic soda and addition of ammonium chloride, when the xanthine remains in solution.

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[1] A mono-methylxanthine has recently been found in the urine after taking much tea and coffee.

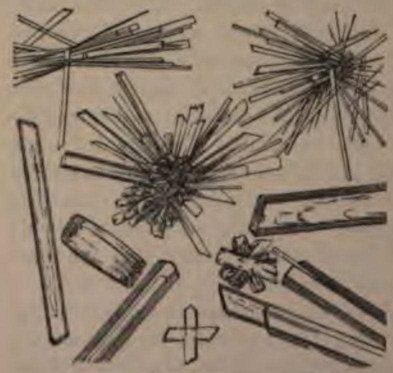

Fig. 16.—GUANINE HYDROCHLORIDE (after Kühne).

Wolff states that the guanine exists in guano partly as a calcium compound and partly in the form of substances like nuclein. From these it is liberated by the preliminary treatment with hot sulphuric acid.

Guanine forms a white amorphous powder which may be heated to 200° without change. It is insoluble in water, alcohol, or ether. Guanine is distinguished from xanthine and hypoxanthine by its insolubility in hot dilute ammonia. It forms crystallisable salts with the stronger acids, and *guanine hydrochloride*,

$C_5H_5N_5O,HCl$+aqua and *guanine nitrate*, $2B,HNO_3 + 3$ aqua, have a characteristic microscopic appearance. The silver nitrate compound of guanine is described on page 307.

On adding a cold saturated aqueous solution of picric acid to a warm solution of guanine hydrochloride, **guanine picrate**, $B,C_6H_3(NO_2)_3O+H_2O$, is thrown down as a highly insoluble precipitate of orange-yellow silky needles. Adenine is the only other base of the xanthine group which is precipitated by picric acid from dilute solutions, but guanidine gives a similar reaction.

Potassium bichromate throws down from solutions of guanine a highly insoluble, orange-red, crystalline precipitate of **guanine dichromate**, $B,H_2Cr_2O_7$. Potassium ferricyanide produces a brown, crystalline precipitate. Xanthine and hypoxanthine give no similar reactions.

Guanine is converted into xanthine by treatment with nitrous acid (compare page 312).

When boiled with strong hydrochloric acid, guanine is decomposed according to the equation :—

$$C_5H_5N_5O+7H_2O=C_2H_5NO_2+4NH_3+CH_2O_2+2CO_2.$$

EPIGUANINE is the name given by M. Krüger (*Chem. Centralb.*, 895, i. 292) to a base isolated from the urine of insane persons. It contains $C_{10}H_{13}N_9O_2$, and resembles guanine. A small quantity of a second base was obtained from the mother-liquors of epiguanine.

Hypoxanthine. Sarkine. $C_5H_5N_4O$.

This base differs from xanthine by an atom of oxygen (see page 306). It separates from its solutions as a white crystalline powder, soluble in 300 parts of cold or 78 of boiling water, and in 900 parts of boiling alcohol. The base is insoluble in ether.

Hypoxanthine forms soluble crystallisable salts with acids. The microscopic appearances of the *nitrate* and *hydrochloride* are characteristic (fig. 17). The *urate*, which is polymeric with xanthine, is precipitated on adding potassium urate to a solution of hypoxanthine hydrochloride.

The silver oxide compound of hypoxanthine, $Ag_2O,C_5H_5N_4O$, is formed as a gelatinous precipitate on adding ammonio-nitrate of silver to an ammoniacal solution of the base. It is insoluble in ammonia, unless used in great excess, and it dissolves with difficulty in boiling nitric acid of 1·10 specific gravity. On cooling, a compound of the formula $C_5H_5N_4O,AgNO_3$ separates in crystals, which, under the microscope, appear as long prisms or spindles, sometimes isolated but in other cases crossed symmetrically to form stellate groups. The last form is common when the crystallisation occurs slowly. The characters of the silver nitrate compound allow of the separation of hypoxanthine from other bases of the group (see page 306).

Hypoxanthine gives negative or only very faint reactions with Strecker's, Weidel's, and Hoppe-Seyler's tests (page 314). After treatment with hydrochloric acid and zinc it gives a ruby-red coloration on addition of caustic soda in excess. In this reaction it behaves like adenine.

Hypoxanthine is almost always associated with xanthine. It occurs in the flesh and muscles of the heart of the horse and ox, in the pancreas, the spleen, and the liver, especially in cases of yellow atrophy. It has also been found in human a dog's urine. It may be isolated by the method described in t table on page 299, and purified by solution in hot water, wi addition of hydroxide of lead, filtration, separation of the le as sulphide, and concentration of the filtrate to the crystallisi point.

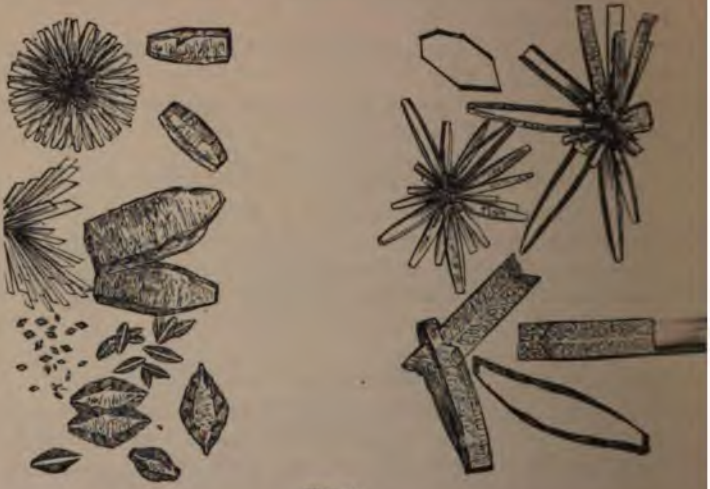

Fig. 17.

HYPOXANTHINE NITRATE. HYPOXANTHINE HYDROCHLORIDE

EPISARKINE, $C_4H_6N_3O$, was obtained by P. Balke (*Jour. pra Chem.*, [2], xlvii. 537), together with hypoxanthine, when extra ing the latter from urine by Salomon's method (see *Jour. Chem. S* lii. 737). It resembles adenine, except for its great insolubili in cold water. It crystallises in prismatic needles, and is be separated from hypoxanthine by dissolving the bases in dilute ammonia as possible, and saturating the solution with carbon dioxide, when the episarkine crystallises out. The hydrochloride crystallises readily. The silver nitrate compound is insoluble in dilute nitric acid, but soluble in ammonia. Episarkine gives no reaction with Weidel's test, but when evaporated with hydrochloric acid and potassium chlorate it yields a white residue which becomes intensely violet on exposure to ammonia. Episarkine is precipitated by phospho-tungstic acid, mercuric chloride, and ammoniacal lead acetate.

Adenine. Imido-sarkine. $C_5H_5N_5$.

Adenine was originally obtained by Kossel in treating pancreas for the preparation of hypoxanthine, but is most conveniently prepared from tea. It contains an alloxan-nucleus, and has the constitution of an imido-hypoxanthine. Hence adenine bears the same relation to that body that guanine does to xanthine (see page 306). Adenine may be converted into hypoxanthine by treatment with nitrous acid.

When pure, adenine crystallises from its aqueous solution in needles, which dissolve in 1086 parts of cold water, and are readily soluble in hot water. It is but slightly soluble in hot alcohol, and is insoluble in ether.

Adenine may be obtained in four-sided pyramids, free from water of crystallisation, by adding excess of ammonia to a concentrated solution of its hydrochloride.

Adenine yields crystallisable salts with acids, and also forms definite compounds with some neutral salts.

Adenine does not give the ordinary colour-reactions characteristic of the xanthine bases, but resembles hypoxanthine in yielding a red coloration on treatment with hydrochloric acid and zinc with subsequent addition of an alkali.

An aqueous solution of adenine ($\frac{1}{2}$ per cent.) gives no precipitate with potassium ferrocyanide or ferricyanide until acetic acid is added, when thin crystalline plates are deposited. With chromic acid adenine forms the compound $(C_5H_5N_5)_2,H_2Cr_2O_7$, crystallising in six-sided plates. Cupric sulphate produces in adenine solutions an amorphous greyish-blue precipitate, consisting of a mixture of copper-adenine and of adenine-copper sulphate. Ferric chloride gives a red coloration unaltered by heat.

Adenine and hypoxanthine combine in aqueous solution to form a compound containing $C_5H_5N_5,C_5H_5N_4O+3H_2O$, which crystallises from water in clusters of slender needles which readily effloresce, and rapidly lose water at 100°. The compound forms a homogeneous hydrochloride, which may be separated into its constituents by dissolving it in dilute sulphuric acid and fractionally crystallising.

For the separation of adenine from the allied bases, G. Bruhns (*Ber.*, xxiii. 225; abst. *Jour. Chem. Soc.*, lviii. 534) employs the following process:—Silver nitrate is added to the nitric acid solution of the bases, when the silver nitrate compounds adenine and hypoxanthine are precipitated, and xanthine and guanine remain in solution. The precipitate is decomposed by sulphuretted hydrogen or dilute hydrochloric acid, the resulting solution nearly neutralised by sodium carbonate, and a solution of sodium picrate added. After standing fifteen minutes, the precipitate of adenine picrate is filtered off, washed, dried at 100°, and weighed. The original precipitate contains $C_5H_5N_5,C_6H_2(NO_2)_3.OH+H_2O$, but becomes anhydrous at 100°, and undergoes no further change below 220°. Adenine picrate is soluble in 3500 parts of cold water, so that a correction of 2·2 milligrammes must be made for every 100 c.c. of filtrate and wash-water. From the filtrate the hypoxanthine is precipitated by neutralising with ammonia and adding ammonio-nitrate of silver. No correction for solubility need be made if both solutions are free from excess of ammonia.

Adenine is very completely precipitated by cupric sulphate in presence of a reducing agent. By employing sodium thiosulphate as the reducing body, and operating in a cold solution, separation from hypoxanthine can be effected (see page 309).

Carnine. $C_7H_8N_4O_3$.

Carnine crystallises in agglomerations of minute irregular crystals. It is very little soluble in cold water, but readily in hot, separating again on cooling. It is insoluble in alcohol or ether. Carnine forms crystallisable salts both with acids and bases. Its hydrochloride gives a golden-yellow precipitate with platinic chloride. The compound with basic lead acetate dissolves in boiling water. The silver nitrate compound resembles that of hypoxanthine, and in its reaction with Weidel's test carnine also behaves like hypoxanthine.

Bromine-water is decolorised when added to a boiling solution of carnine. On concentrating the liquid at 100°, brilliant needles of hypoxanthine hydrobromide are deposited, and on treatment with caustic soda yield the free base.

The occurrence of carnine in urine is doubtful. Hitherto it has only been found with certainty in extract of meat, of which it constitutes about 1 per cent.

)MAÏNES.

ıe ptomaïnes are bodies analogous to the vegetable alkaloids,
ıced in the putrefactive decomposition of animal tissues and
: nitrogenous organic matters. Their formation may occur in
g tissues as well as after death, but they are in all cases the
ıcts of bacterial life. Contrary to the general impression,
ıajority of known ptomaïnes are destitute of marked poisonous
ırties.

ıe term ptomaïne (πτῶμα, cadaver or corpse; ινο, material)
e to Selmi, whose researches (1873 to 1876) were among the
ıst on the alkaloids of putrefaction, and first directed general
tion to the subject. Selmi showed that different ptomaïnes
be obtained by extracting solutions of putrid matter succes-
with ether, chloroform, and amylic alcohol, and that the fluid
etained ptomaïnes which were not extracted by any of these
nts. The ptomaïnes were thus proved to be numerous, and
of them were found to give reactions simulating those of the
-bases. Selmi operated with extracts only, and did not
e any of his bases in a pure state. Nencki (1876) was the
chemist to obtain a ptomaïne in a state sufficiently pure to
its composition to be ascertained. It was found to contain
$_1$N, and hence to be isomeric with collidine, from which,
ver, it differed in many respects. Since then, many other
aïnes have been isolated and analysed, but much remains to be
ed respecting their constitution, chemical characters, reactions,
hysiological action.[1]

ıny writers on the subject of ptomaïnes have adopted un-
ioned as generally true the description of such of them as

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nimal bases are being constantly produced in the animal system as the
of normal physiological processes, and, in ordinary circumstances,
gularly eliminated by the kidneys, bowels, skin, and lungs; but when,
ıny cause, one or more of these channels of elimination becomes ineffec-
ın accumulation of the effete matters occurs in the system, and a toxic
is exerted by them on the nerve-centres. The headache associated with
pation is ascribable to this cause, and more serious nervous symptoms
from the deficient excretory action of the kidneys which occurs in certain
es of those organs. In addition to being got rid of by actual excretion,
ete matters are also largely destroyed by oxidation, the liver apparently
g a leading part in this action. The fever of prostration, which results
ırfectly healthy body from over-exertion, is due to accumulation of waste
s which have been found in excess, and but imperfectly eliminated or
ed. In the case of the infectious fevers, it is probable that the
oms are due to the toxic action of ptomaïnes produced by specific
organisms which have been introduced into the system, and found it
le for their development.

were known to the earlier observers, and have regarded their chemical relationships as being much closer than is actually the case. In many instances, observers have assumed ptomaïnes to be absent from putrefying material, because no substance of basic character could be extracted therefrom by ether or chloroform, ignoring or being ignorant of the fact that a number of the best-known ptomaïnes are not extracted from aqueous liquids by such solvents. Similarly the reduction of ferric ferricyanide to prussian blue, observed by Brouardel and Boutmy to be produced by certain ptomaïnes, and erroneously supposed by these chemists to be peculiar to and characteristic of such bases, has been assumed by later observers to be produced by bases having no analogy with or relationship to those which were found to give the reaction, except the very distant one of being also products of putrefactive decomposition. Nor does it appear to have been generally recognised that the striking difference in the nature of the ptomaïnes isolated by Gautier, Nencki, and others (collidine, hydrocollidine, hydrolutidine, &c.), and those found by Brieger (cadaverine, neuridine, putrescine, &c.), was due to the method of extraction employed, the former being obtained from an ether or chloroform extract, and the latter by precipitation from an aqueous liquid.

Chemistry of Putrefaction.

The chemistry of putrefactive decomposition has made great advances of late years, and is now well understood to signify the process of fermentation that nitrogenous organic matters, especially proteids, undergo in presence of living bacteria at a suitable temperature.

The putrefaction of proteids usually commences by the transformation of the proteid substance itself, first into albuminates, that is, bodies soluble in liquids of acid or alkaline reaction, but precipitated on neutralisation; secondly, into peptones, which are proteid bodies soluble at all temperatures in all aqueous liquids, whether acid, alkaline, or neutral. The second stage in putrefaction consists in the breaking up of these proteid bodies to form compounds of comparatively simple and definite composition, of which the principal are leucine, tyrosine, and indole. The two latter bodies belong to the aromatic series, the members of which usually if not invariably possess more or less marked antiseptic properties, that is, they are antagonistic to the life of ferment-organisms.

In the process of putrefaction the initial material is proteid, and the end-products are ammonia and carbon dioxide, so that the difference between putrefactive change and the normal decoms which occur in the living animal are chiefly in the diate stages, the formation of glucosides of fatty acids specially characteristic of living processes, while the pro- of amido-derivatives of the lower fatty acids characterises tive decomposition.

876, E. Salkowski, when investigating the condition of ne in several cases of *ileus*, in which an indigo-producing as produced in excessive amount, found that on distilla- h hydrochloric acid the urine yielded a notable quantity nol. This was subsequently proved to exist in the urine orm of a salt of phenyl-sulphuric acid, $(C_6H_5)HSO_4$ rt i. page 9), isomeric with phenol-sulphonic acid, $O_3H)OH$; and the same body, or its homologue cresyl- ric acid, $(C_7H_7)HSO_4$, appears to be constantly present rine of the horse.

study of indole and skatole, two bodies characteristic of ed to the recognition of their relation to phenol, which nd was found to be excreted most abundantly by patients g from septic diseases, particularly suppurating wounds, ing empyæmias, &c., whence it appeared that "that sub- (phenol) which is generally regarded as antiseptic *par* *e* is itself a product of putrefaction."

until a comparatively recent period tyrosine was the own representative of the aromatic group among the pro- f septic or intestinal decompositions of proteids. The of tyrosine to phenol is indicated by the constitution of ner body as a phenyl-amidohydoxylactic acid, and by the t phenol can be actually obtained from tyrosine by the of caustic potash ; and this suggested that the phenol g from bacterial decomposition was not derived primarily e proteid molecule, but secondarily through the decomposi- tyrosine. By treating an aqueous solution of tyrosine fragment of putrid pancreas, and allowing it to ferment for ys, an abundant yield of hydroparacoumaric acid ained, of which tyrosine may be regarded as the amido- ve. The bacteria appear to act by causing a production ent hydrogen, which then decomposes the tyrosine with n of ammonia. The further stages of the change have perimentally verified by Baumann by treating ammonium racoumarate with sewage-water and allowing the liquid to . After ten days, phenol, paracresol, and oxyphenylacetic re recognised among the products of the decomposition, ious stages of which may be represented by the following s :—

Tyrosine, ... $C_6H_4 \begin{cases} CH_2.CH(NH_2).COOH \\ OH \end{cases}$ *plus* $H_2 - NH_3 +$

Hydroparaparacoumaric acid, $C_6H_4 \begin{cases} CH_2.CH_2.COOH \\ OH \end{cases}$; which, *plus* $H_2 - CH_4 +$

Hydroxyphenyl-acetic acid, $C_6H_4 \begin{cases} CH_2.COOH \\ OH \end{cases}$; which, *plus* $H_2 - CH_4 +$

Para-hydroxybenzoic acid, $C_6H_4 \begin{cases} COOH \\ OH \end{cases}$; which, *minus* $CO_2 -$

Phenol, ... $C_6H_4 \begin{cases} H \\ OH \end{cases}$

If CO_2 be split off at any earlier stage, paracresol, $C_6H_4(CH_3)OH$, results instead of phenol.

It is evident that the foregoing reactions may, and probably will, proceed simultaneously, in which case we have the following changes:—

$$\underset{\text{Tyrosine.}}{C_6H_4(OH).CH_2.CH(NH_2).COOH} + H_2 = \underset{\text{Hydroxyphenyl-acetic acid.}}{C_6H_4(OH).CH_2.COOH} + \underset{\text{Methylamine.}}{CH_3.NH_2}; \text{ or}$$

$$\underset{\text{Tyrosine.}}{C_6H_4(OH).CH_2.CH(NH_2).COOH} + H_2 = \underset{\text{Paracresol.}}{C_6H_4(OH).CH_3} + \underset{\text{Glycocine.}}{CH_2(NH_2).COOH}; \text{ or,}$$

$$\underset{\text{Tyrosine.}}{C_6H_4(OH).CH_2.CH(NH_2).COOH} + H_2 = \underset{\text{Phenol.}}{C_6H_4(OH).H} + \underset{\text{Sarcosine.}}{CH_2(NH_2).CH_2.COOH}.$$

Concurrently with the formation of tyrosine, indole, &c., from bodies containing an aromatic nucleus proceeds the formation of leucine, $C_6H_{13}NO_2$, and other amido-fatty acids from compounds of the fatty series, while the lecithins split up with formation of choline, neurine, &c. These bodies have long been known as products of putrefactive change, but it is only comparatively recently that other bases apparently peculiar to such conditions have been recognised. To these bases the name of ptomaïnes or cadaveric alkaloids is more properly applied, and might be advantageously restricted.

The formation of ptomaïnes during the putrefaction of albuminous substances appears to follow certain laws, and bases which are comparatively abundant during the earlier periods disappear entirely or partly, and are replaced by others during the subsequent stages.

Brieger points out that lecithin (page 241) begins to decompose almost immediately after death, with formation of choline and distearyl-phospho-glyceric acid (phospho-distearin)[1]:—

$$(C_{18}H_{35}O.O)_2:C_3H_5.OPO(OH).O.C_2H_4N(CH_3)_2.OH + OH_2 =$$
$$(C_{18}H_{35}O.O)_2:C_3H_5.OPO(OH)_2 + OH.C_2H_4.N(CH_3)_2.OH$$

Hence, in the incipient stage of putrefaction, choline is the only ptomaïne which can be detected, but other bases are rapidly formed as the decomposition progresses, neuridine being especially prominent about the third day. This base appears to be one of the most constant products of putrefaction, and is always accompanied by choline; but while the latter substance gradually disappears with the progress of the decomposition, and is replaced by trimethylamine, the neuridine augments in quantity, day by day, up to a certain point, when it also gradually decreases and ultimately disappears entirely.[2] Brieger detected neuridine on the third day of putrefaction of pancreas and liver. The choline had disappeared completely by the seventh day, but neuridine was detected until the fourteenth day.

Brieger has further remarked that highly poisonous ptomaïnes are never formed during the earlier stages of the putrefaction of human organs. The formation of a highly toxic ptomaïne corresponds with the disappearance of the choline. Trimethylamine appears at about the same time, being probably a product of the decomposition of the choline, and possibly also of the neuridine.

After the formation of neuridine the isomeric base cadaverine appears, and continues to augment throughout the putrefaction, while a second isomer, saprine, and the bases putrescine and mydaleine also make their appearance.

The three isomeric ptomaïnes, cadaverine, putrescine, and saprine, are not poisonous, but mydaleine, the chloroplatinate of which contains C, 10·83; H, 3·23; and Pt, 38·74 per cent., is a very poisonous diamine.

All these four bases have been detected in human corpses. Along with them has been found another highly poisonous ptomaïne, the chloroplatinate of which contains 41·30 per cent. of

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[1] The body formulated in the text has the constitution of a distearin-lecithin; but analogous bodies have been isolated containing an oleic and palmitic radical in the same molecule, instead of two stearyl groups (see page 242).

[2] The quantity of neuridine formed appears to depend on the nature of the organs undergoing putrefaction, it being produced most abundantly by the intestines and glandular organs, and only in very small amount by the liver and spleen.

Pt, while a second, apparently innocuous, which boils at 284° C., remains in the mother-liquors.

It appears from Brieger's researches that the same species of bacteria give different products, according to the soil in which they are cultivated. Neurine is the leading ptomaïne produced by the bacterial fermentation of the flesh of mammals, while in examining putrefying fish, Brieger met with a base having the same composition and physiological action as muscarine, $C_5H_{15}NO_3$, or oxyneurine (page 245). The putrefaction of cheese yielded neuridine; that of glue gave neuridine, a base allied to muscarine, and isophenylethylamine.

The bacillus to which Eberth attributes typhoid fever is not produced in putrefaction; but there are found in the evacuations traces of a base which dilates the pupil, produces diarrhœa, and is rapidly fatal to animals.

The study of the ptomaïnes is seriously complicated by the fact that many of the bodies described have been isolated by medical men using empirical methods and having neither the time nor the capacity to make a thorough examination of the bases obtained, or to compare them with similar products described by other observers. Hence it is probable that many of the ptomaïnes which have been described and regarded as definite individuals are in reality impure known products or mixtures of two or more such bodies.

Poisonous Food.—As ptomaïnes, some of which are toxic, are frequently formed in the putrefaction of proteids, they are liable to exist in decomposing food. Some of the most violent attacks of ptomaïnic poisoning on record have resulted from partaking of food in which the putrefactive change was so slight as not to attract attention in any way.

The symptoms resulting from eating poisonous food usually commence with nausea, vomiting, pain in the abdominal region, and diarrhœa, the motions being frequently of an offensive character. The nervous symptoms are commonly faintness, muscular weakness, prostration, and sometimes spasms; followed by fever, headache, and thirst. Cramps, clonic spasms, dilatation of the pupil, and disturbance of vision, with drowsiness and occasionally coma, are also observed. The *post-mortem* appearances are similar to those produced by mineral irritant poisons. The effects of ordinary poisons are commonly manifested within three or four hours after partaking of the poisoned food, and often in a much shorter time; the symptoms of ptomaïnic poisoning rarely become fully developed in less than six to eight hours, and in some cases are much longer delayed. But in such cases the delay is probably due to the time required for the multiplication of the micro-organisms, and points to the great probability of the toxic ptomaïne which they produce being formed after the food was ingested.

Detection and Isolation of Ptomaïnes.

The following description of the physical and chemical characters of ptomaïnes is compiled from the works of Brieger and others, but it must be borne in mind that no statements can be generally true of bodies of such diverse nature.

In their physical and chemical characters the ptomaïnes present a close general resemblance to the plant-bases; and, like these, may be differentiated into volatile, liquid, oxygen-free bases, and non-volatile, solid, oxygenated bases.

The liquid ptomaïnes have a penetrating and persistent odour, which is often nauseous or cadaveric. In other cases, their odour recalls that of seringa, hawthorn, or musk. This smell is so persistent that Gautier observed it in products of ancient putrefactions, transformed into guano and calcium phosphate, met with in a bone-cavern of the Stone Age. All the liquid ptomaïnes are soluble in ether-alcohol, and some of them in chloroform and amylic alcohol.

The solid ptomaïnes are generally crystallisable, colourless, very soluble in water, but insoluble in alcohol, chloroform, or benzene. In presence of impurities these characters are susceptible of considerable variation.

Many of the ptomaïnes are powerful bases, having a strong alkaline reaction, neutralising acids perfectly to form well-defined salts, and in some cases even absorb carbon dioxide from the air. In many cases they yield striking colour-reactions.

Many of the ptomaïnes are very unstable and susceptible of oxidation, so that the evaporation of solutions containing them should, as far as possible, be conducted *in vacuo*, and at a temperature not exceeding 40° to 50° C.

As a consequence of their proneness to undergo oxidation, many ptomaïnes reduce, either in the cold or on heating, auric chloride, platinic chloride (used in excess), silver nitrate, ferric chloride, iodic acid, chromic acid, &c.

The ptomaïnes yield precipitates with the majority of the general reagents for alkaloids, but the reactions are rarely characteristic.

With platinic chloride the hydrochlorides of the ptomaïnes usually yield soluble chloroplatinates, which are very frequently crystallisable. An excess of the reagent in many cases decomposes them.

Mercuric chloride precipitates most of the ptomaïnes from sufficiently-concentrated aqueous solutions, and more generally and perfectly from alcoholic solutions. The compounds formed may be crystallised from their solutions in boiling water.

Most ptomaïnes yield soluble salts with auric chloride, or else produce precipitates soluble in boiling water, which are readily decomposed with separation of metallic gold.

Picric acid and tannin yield insoluble or sparingly soluble precipitates with the majority of ptomaïnes.

Phospho-molybdic acid is the only reagent which has been found to precipitate all the ptomaïnes without exception.

Selmi (1878) has described the following colour-reactions of ptomaïnes, but it is doubtful how far the effects observed are attributable to impurities, and how far the reactions are common to all ptomaïnes:—Concentrated sulphuric acid, when added cautiously to a ptomaïne, produces a violet-red coloration. Hydrochloric acid (preferably mixed with a little sulphuric acid) gives a violet-red colour, which becomes more pronounced on heating. When a ptomaïne is heated for some time with nitric acid, and excess of caustic potash then added, a fine golden-yellow coloration is produced. Some ptomaïnes give with iodic acid a reaction similar to that of morphine (Part ii. page 318).

Various attempts have been made to establish some sharp distinction between ptomaïnes and plant-bases, but hitherto without success.[1] Many of the vegetable alkaloids are optically active, which the ptomaïnes, as a rule, are not, but the quantity of the material ordinarily available for experiment is quite insufficient to render such a character of practical value. Many of the ptomaïnes are powerful reducing agents, and Brouardel and Boutmy (1881) proposed to distinguish them by the immediate reducing action they exerted on a mixture of potassium ferricyanide and ferric chloride solutions, with formation of prussian blue. But Brieger found that immediate reduction of the reagent, with production of a blue colour, was produced by cadaverine, saprine, mydaleine, and certain other ptomaïnes; but not by neuridine, putrescine, or choline. On the other hand, of the vegetable alkaloids, morphine, veratrine, and colchicine immediately reduce the reagent, while a blue coloration is developed slowly and feebly in presence of aconitine, brucine, codeine, conine, narceine, nicotine, papaverine, strychnine, &c.

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[1] It is not to be expected that a class of bodies of such varying characters as the plant-bases should exhibit any one marked feature of distinction from the ptomaïnes, which are themselves equally diverse in characters and constitution.

For the extraction of ptomaïnes from animal matters, methods are employed closely resembling those used for the isolation of the vegetable alkaloids (Vol. iii. Part ii.), the modification of the Stas-Otto process used for the separation of strychnine being especially suitable. But all evaporations should be conducted at as low a temperature as possible, and the solvents should have been previously treated with a little hydrochloric acid, and redistilled to separate traces of pyridine or other bases which might otherwise be mistaken for ptomaïnes. This is specially important in the case of amylic alcohol, which is very liable to contain traces of volatile bases, and there is no doubt that these have been mistaken for ptomaïnes extracted from the animal matters under treatment.

The methods of isolating the ptomaïnes of the neuridine class are described under "Diamine-ptomaïnes" (pages 334, 336, 340).

Gautier recommends the following process for the isolation of the volatile ptomaïnes with which his name is so closely associated:—The liquid to be examined is rendered distinctly acid with oxalic acid, warmed, filtered, and distilled in a vacuum. The distillate will contain any volatile fatty acids, phenols, pyrrol, indole, and skatole. The residue in the retort is treated with a moderate excess of milk of lime, filtered, and distilled in a vacuum, the distillate being received in dilute sulphuric acid. The distillate is rendered exactly neutral by ammonia, evaporated nearly to dryness, and the crystals of ammonium sulphate, &c., which form, separated from the mother-liquor. This is further concentrated, treated with absolute alcohol, and the liquid, which contains in solution any ptomaïnes as sulphates, is filtered and evaporated to a small bulk. The residual liquid is treated with caustic soda, and the bases extracted by agitating it successively with ether, petroleum-ether, and chloroform.

Owing to the close similarity in properties between the ptomaïnes and plant-bases, great difficulty sometimes exists in distinguishing with certainty the origin of an alkaloidal body extracted from putrefying material. In toxicological inquiries this difficulty becomes of the first importance, though it is liable to great exaggeration by those interested in proving the innocence of persons accused of committing murder by poisoning. But in several authentic cases on record, ptomaïnes produced by putrefactive decomposition after death have undoubtedly been mistaken for poisonous vegetable alkaloids administered during life, and hence too great care cannot be taken by the toxicologist to exclude from analytical operations processes by which ptomaïnes might be formed, and to identify the alkaloidal substance extracted in every way in his power. The formation of ptomaïnes may be prevented by immersing the animal tissues to be examined in alcohol, which prevents putrefactive decomposition without interfering with the subsequent examination for alkaloids. According to Coppola the formation of ptomaïnes is caused or promoted by digesting animal matters with acids, especially when the mixtures are acidulated with sulphuric acid. In practice, there is never any occasion to use such a reagent, acetic acid being free from the same objection, and more suitable in other respects.

Of the ptomaïnes formed in corpses, and therefore likely in practice to be mistaken for vegetable alkaloids, by far the greater number were discovered by Selmi. These, with others, have been classified by Husemann in the following five groups, according to their behaviour with solvents:—

1. *Ptomaïnes which are extracted by ether from acid solutions.*—Selmi isolated the bases of this group from corpses. Their behaviour toward tannic acid, iodised iodide of potassium, and auric chloride was similar to that of the natural vegetable alkaloids. Mercuric chloride gave no precipitate.

On evaporating two or three drops of the aqueous solution, the addition of three drops of hydrochloric acid and one drop of sulphuric acid produced, on warming, a beautiful violet colour. Nitric acid coloured it yellow. Ptomaïnes of this class may be mistaken for digitalin, which is also taken up by ether from acid solutions. But, according to Selmi, no ptomaïne which is extracted from acid solutions by ether will give the characteristic reaction of digitalin with bromine and sulphuric acid.

2. *Ptomaïnes which are extracted by ether from alkaline solutions.*—According to Selmi, the ptomaïnes belonging to this group give various colour-reactions, and they form crystalline salts. When subjected to physiological tests, they generally occasion dilation of the pupil of the eye, and as a rule a diminution of the frequency of respiration. Most of these ptomaïnes reduce iodic acid like morphine, and give with phospho-molybdic acid at first a violet and afterwards a blue coloration. They reduce auric chloride and a mixture of potassium dichromate with sulphuric acid. Most of the ptomaïnes of this group are not precipitated by platinic chloride. They include the greater number of cadaveric alkaloids.

3. *Ptomaïnes not soluble in ether but soluble in chloroform, as obtained from alkaline solutions.*—Selmi described the bases of this group as possessing a strong alkaline reaction, having a pungent, more or less bitter taste, and readily decomposing on the evaporation of their chloroformic solution, even at a low tempera-

ture, the residue becoming partly insoluble in chloroform. All of the bases of this group reduce iodic acid, and frequently form crystalline compounds with iodised potassium iodide. They give red colorations with sulphuric acid, and similar reactions with Fröhde's test.

4. *Ptomaïnes insoluble in ether and in chloroform, but which are readily extracted from alkaline solution by amylic alcohol.* Of these bases Selmi has observed several, one not poisonous, the others poisonous. He mentions an instance where he obtained a ptomaïne which formed long needle-shaped crystals with hydriodic acid. Another illustration is given by Felesar, who, in the judicial investigation of a corpse, extracted a base by means of amylic alcohol which did not reduce iodic acid and gave no colour-reaction with the usual tests for plant-bases.

Amylic alcohol removes morphine from alkaline solutions after their extraction with ether and chloroform, and hence may be referred to group four. There are comparatively few ptomaïnes belonging to this group, and these do not exhibit many of the reactions belonging to morphine, and are frequently entirely absent. The colour-reactions for morphine are many and well-established, and no cadaveric alkaloid simulates all of them (Part ii. pp. 303, 313).

5. *Ptomaïnes which are not extracted either by ether, chloroform, or amylic alcohol.*—These bases are very soluble in water, and almost tasteless. They give no colour-reaction with sulphuric acid, and are not precipitated by mercuric chloride, auric chloride, or hydriodic acid.

Classification of Ptomaïnes.

The known ptomaïnes are so numerous and of such varied constitution that it is impractical, as it is unscientific, to attempt to discover any characters or reactions common to all except those inseparable from their basic nature. A more promising method is to classify them as far as possible according to their chemical constitution, placing in a group apart those of which the constitution is still unknown; and then, as more accurate knowledge is obtained concerning the latter, to transfer them to their proper classes, or create new classes as occasion may arise. Proceeding on this plan, the ptomaïnes of which the constitution is known may be conveniently grouped as in the following table (pages 332, 333).

Further information respecting some of these ptomaïnes will be found under their respective heads, while a more detailed description of the ptomaïnes which remain unclassified, and hence do not appear in the table, is given in the sequel.

Name and Constitution.	Formula.	Observer.	Source or Mode of Formation.	Physiological Action and other Characters.
PYRIDINE DERIVATIVES.				
Isocollidine. Isophenyl-ethylamine,	$H_2N.CH.MePh$	Nencki.	Putrefying gelatin and ox pancreas.	Part ii. page 109.
Isoparvoline,	$C_9H_{13}N$	Gautier and Etard.	Putrefying mackerel and horse-flesh.	Very poisonous. Oily base, boiling at 180°.
Isocoridine,	$C_{10}H_{15}N$	Guareschi and Mono.	Putrefying fibrin.	Part ii. page 97.
Hydrolutidine,	$C_7H_{11}N$	Gautier and Mourgues.	Cod-liver oil.	Paralysis and death. Colourless alkaline oil.
Hydrocollidine,	$C_8H_{13}N$	Gautier.	Putrefying mackerel, horse-flesh, &c.	Tetanic convulsions; death. Colourless liquid, smelling of *syringa*.
Hydrocorridine,	$C_{10}H_{17}N$	Griffiths.	Cultivations of *Bact. allii* on nutrient agar-agar.	Poisonous. White solid; odour of hawthorn.
MONOMINES.				
Trimethylamine,	$(CH_3)_3N$	... ...	Herring brine. Putrid corpses.	Part ii. page 12.
Triethylamine,	$(C_2H_5)_3N$	Brieger and others.	Putrefied animal substances.	Part ii. page 17.
Propylamine,	$(C_3H_7)H_2N$	Brieger.	Putrefied animal substances.	... ...
Butylamine,	$(C_4H_9)H_2N$	Gautier and Mourgues.	Cod-liver oil.	Poisonous.
Amylamine,	$(C_5H_{11})H_2N$	Gautier and Mourgues.	Cod-liver oil.	Poisonous.
Hexylamine,	$(C_6H_{13})H_2N$	Gautier and Mourgues.	Cod-liver oil.	Poisonous.
DIAMINES.				
Ethidine-diamine,	$CH(CH_3)(NH_2)_2$	Brieger.	Putrid haddock.	Poisonous. Page 334.
Ethylene-diamine,	$C_2H_4(NH_2)_2$	Brieger.	Putrefying cod-fish.	Not poisonous. P. 194.
Diethylene-diamine,	$H_2N.C_2H_4.C_2H_4.NH_2$	Schreiner.	In semen (?). Cultivations of tubercle bacillus.	Page 194.
Trimethylene-diamine,	$H_2N(CH_2)_3NH_2$	Brieger.	Putrefying beef-broth.	Poisonous.
Putrescine. Dimethyl-ethylene-diamine,	$MeHN.C_2H_4.NHMe$	Brieger.	With cadaverine, &c.	Page 341.
Cadaverine. Pentamethylene-diamine,	$C_5H_{14}N_2$	Brieger.	Constant product of putrefaction of proteids.	Not poisonous when pure. Page 339.
Neuridine,	$C_5H_{14}N_2$	Brieger.	Constant product of putrefaction of proteids.	Not poisonous when pure.
Saprine,	$C_5H_{14}N_2$	Brieger.	Putrefying spleen and liver.	Not poisonous. P. 341.
Mydaleine,	... ...	Brieger.	Formed with cadaverine and neuridine.	Paralysis; death. Page 342.
Hexamethylene-diamine,	$C_6H_{16}N_2$	Garcia.	Putrefying horse-flesh and pancreas.	Page 334.

AMIDO-ACIDS AND BETAÏNES.				
Sarcosine. Methyl-glycocine,	$C_3H_7NO_2$	Various.	Putrefying proteids.	Page 233.
δ-Amidovaleric Acid,	$C_5H_{11}NO_2$	Salkowski, Gabriel and Ashan.	Putrid fibrin.	... ...
Leucine. α-Amido *n*-Hexoic Acid,	$C_6H_{13}NO_2$	Proust, Salkowsky, &c.	Putrefying cheese, proteids, and gelatin; diseased liver; urine in cases of typhus, small-pox, phosphorus poisoning.	Page 211.
Tyrosine,	$C_9H_{11}NO_3$	... ...	With leucine.	Page 216.
Betaïne. Trimethyl-glycocine,	$C_5H_{11}NO_2$	Liebreich, Brieger.	From urine. Poisonous and non-poisonous mussels.	Not poisonous. Page 234.
Neurine,	$C_5H_{13}NO_2$	Brieger.	Constant product of putrefaction of proteids.	Paralysis. Page 236.
Choline,	$C_5H_{15}NO_2$	Brieger.	Constant product of putrefaction of proteids.	Paralysis. Page 237.
Muscarine,	$C_5H_{15}NO_3$	Brieger.	Putrid fish. In poisonous toadstools.	Intensely poisonous. Page 245.
Isomuscarine,	$C_5H_{15}NO_3$	... ...	Artificial synthesis.	Moderately poisonous. Page 233.
IMIDO-BASES.				
Dimethylene-imide,	$HN(CH_2)_2$	Schreiner.	In semen (?). Formed by tubercle bacillus.	Not poisonous.
Methyl-guanidine,	$HN{:}C\left\{\begin{matrix}NHCH_3\\NH_2\end{matrix}\right.$	Brieger.	Putrid flesh.	Very poisonous; cholera. Page 285.
Glycocyamidine,	$HN{:}C\left\{\begin{matrix}NH.CH_2\\NH.CO\end{matrix}\right\}$	Griffiths.	Urine in cases of measles.	Very poisonous; high fever, death. P. 282.
Propyl-glycocyamine,	$HN{:}C\left\{\begin{matrix}N(C_3H_7).CH_2.COOH\\NH_2\end{matrix}\right.$	Griffiths.	Urine in mumps.	Poisonous; nervous excitement, convulsions, death.
Indole,	$HN.C_8H_7$	Various.	Intestinal putrefaction; fæces.	Headache, &c.
Skatole,	$HN.C_8H_6(CH_3)$	Various.	Intestinal putrefaction; fæces.	Headache, &c.

Diamine Ptomaïnes.

Some of the most characteristic of the bases produced during putrefactive change have the constitution of diamines. Thus among the products of putrefaction there have been observed:—

Ethidene-diamine,[1] .	$H_2N.CH(CH_3).NH_2$
Ethylene-diamine (page 194), . . .	$H_2N.C_2H_4.NH_2$
Diethylene-diamine (page 194), . . .	$H_2N.C_2H_4.C_2H_4.NH_2$
Dimethyl-ethylene-diamine (putrescine),	$(CH_3)HN.C_2H_4.NH(CH_3)$
Trimethylene-diamine,[2]	$H_2N.CH_2.CH_2.CH_2NH_2$
Pentamethylene-diamine, (cadaverine), . .	$H_2N.CH_2.CH_2.CH_2.CH_2.CH_2.NH_2$
Hexamethylene-diamine,	$H_2N.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.NH_2$

In addition to these diamines, neuridine and several other important ptomaïnes belong to the same class, but their constitution is not exactly known. Meta-phenylenediamine and paraphenylenediamine are not known to be natural products, but are closely related to the diamine-ptomaïnes.[3] They have already been described (Part ii.).

Von Udranszky and Baumann have observed (*Ber.*, xxi. 2744) that when a dilute aqueous solution of a fatty diamine is shaken with benzoyl chloride and caustic soda, the diamine is almost completely converted into an insoluble dibenzoyl-derivative, which is easily separated from benzamide and other nitrogenous products by dissolving the precipitate in alcohol and pouring the solution into a large volume of water, which retains the benzamide in solution. After standing for a short time, the dibenzoyl-derivative crystallises from the solution. The monobenzoyl-derivative med in this reaction. A distinct precipitate is obtained ution of 0·005 gramme of ethylamine-diamine hydrate of water is mixed with a few drops of the reagent, ition containing 0·053 gramme in 100 c.c. of water 3 gramme of the pure dibenzoyl-derivative. Tetra- -diamine and pentamethylene-diamine are precipitated e solutions even more completely than ethylene-diamine, libenzoyl-derivatives can be obtained quite pure by g them from alcohol or ether. A solution of 0·0079 f pentamethylene-diamine in 100 c.c. of water gave amme of the dibenzoyl-derivative when shaken with 5 zoyl chloride and 40 c.c. of a 10 per cent. solution of la, and left for twenty-four hours. In a second similar t, only 0·0142 of the derivative was obtained, so that tative results have but little value. By the above means, ky and Baumann isolated from the urine of a patient, rom cystinuria and inflammation of the bladder, penta- -diamine ("cadaverine"), tetramethylene-diamine ("pu- , and a diamine forming a chloroplatinate more soluble of pentamethylene-diamine. A mixture of diamines, etramethylene-diamine predominated, was also obtained fæces of the patient.[1] Cystin was also almost invari- l in the urine, and it is probable that the amounts of nd of cystin are in some way related. Normal urine and ces contain no trace of diamines.[2]

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[1] ETHIDENE-DIAMINE, isomeric with ethylene-diamine, was found by Brieger in putrid haddock, together with neuridine, gadinine, muscarine, and trimethylamine. It differs from ethylene-diamine in being poisonous, and in forming platinum and gold salts which are more soluble than the corresponding compounds of ethylene-diamine. When injected subcutaneously, ethidene-diamine occasions in guinea-pigs abundant secretion from the mucous membranes, and dilation of the pupil. The eyeballs dilate, there is acute dyspnœa, and death ensues with stoppage of the heart in diastole.

[2] Said to have been isolated by Brieger, from cultivations of the comma bacillus in beef-broth.

[3] In the decomposition of albumin by acids, alkalies, or soluble ferments n diamines are formed, these bases being strictly characteristic of *bacteri* decomposition of proteid matter.

rcia (*Zeit. physiol. Chem.*, xvii. 543, 571) has applied l chloride reaction to the examination of the ptomaïnes putrefying mixtures of horse-flesh and pancreas. He t the production of putrescine, cadaverine, and hexa- -diamine is an early phenomenon in such putrefying at a favourable temperature. It reaches its highest bout three days, and the three diamines named are pro- the same proportion throughout. In cystinuria, tetra- -diamine only is produced in the later stages. Infec-

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zoyl-compounds may be differentiated by recrystallising them from observing their melting-points and comparative solubilities in ether (see page 340).

ann has also suggested the use of benzoyl chloride for the precipi- rbohydrates, and Wedensky has applied the reaction to the traces of glucose and other carbohydrates in normal urine. states that the colouring matter of urine is also precipitated by oride. It appears possible that some confusion might occur recipitate due to carbohydrates or urinary colouring matter and d by diamines.

tion of nutritive media with the fæces of such patients causes the appearance of ptomaïnes.

For the *isolation* of ptomaïnes of the neuridine class (diamines) Brieger recommends the following method, in place of others previously described by him:—The putrefying substance is treated cautiously with hydrochloric acid, which if not used in too great excess appears to increase the stability of the diamine-ptomaïnes. The mixture is brought to a syrupy consistence, and then treated with absolute alcohol. The strained or filtered liquid is evaporated at a low temperature, and the residue taken up with a fresh quantity of absolute alcohol. This solution is filtered and treated with excess of an alcoholic solution of mercuric chloride, and allowed to stand for twenty-four hours. The precipitate is separated by filtration and treated with a large quantity of boiling water, and the liquid filtered. The mercury compounds of peptones and albuminates are left insoluble, while those of the ptomaïnes will be found in the filtrate. The mercury is precipitated from the solution by sulphuretted hydrogen, when the filtered liquid will contain the hydrochlorides of the diamines in a tolerably pure state.

For the further *purification* of the diamine-ptomaïnes, the liquid should be made neutral to litmus by careful addition of soda, concentrated to a syrup, and treated with strong alcohol to precipitate inorganic matters, &c. The alcoholic extract is filtered, evaporated, dissolved in a little water, neutralised with soda, acidulated with nitric acid, and precipitated with phospho-molybdic acid. The precipitate is separated, and decomposed by careful heating with neutral lead acetate. The filtrate is treated with sulphuretted hydrogen to remove the excess of lead, and evaporated to a syrup, when the hydrochlorides of the ptomaïnes will be obtained in a fairly pure state, and can be converted into picrates or the platinum or gold salts of the bases.[1]

For the *separation* of bases of the neuridine group, Brieger precipitates them as mercuri-chlorides, and then has recourse to differences in the solubility of their compounds.

Neuridine may be precipitated as the picrate, which is a compound insoluble in cold water, but soluble on heating.

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[1] "Considerable difficulty in the purification of the ptomaïnes is caused by a nitrogenous, amorphous, non-poisonous, albumin-like substance, which passes into all solutions, and can only be got rid of by careful precipitation with an alcoholic solution of lead acetate, in excess of which reagent the precipitate is soluble. This albuminoid forms an amorphous compound with platinum, and acts as a strong reducing agent (the platinum compound contains 29 per cent. of platinum). When this albuminoid is eliminated, the hydrochlorates or the double salts of the ptomaïnes crystallise."—Brieger.

Putrescine aurichloride is very slightly soluble in water. The hydrochloride crystallises very readily in needles from its solution in rectified spirit.

Cadaverine aurichloride is very soluble. The platinum salt crystallises well and is only very slightly soluble. Saprine chloroplatinate is soluble in water.

Mydaleine hydrochloride is extremely soluble, and remains in the alcoholic mother-liquors from which the hydrochlorides of the other bases have been crystallised.

The reduction of a mixture of ferric chloride and potassium ferricyanide, supposed by Brouardel and Boutmy to be a reaction characteristic of ptomaïnes, is not yielded by choline, neuridine, putrescine, or saprine.

The differing solubilities of the dibenzoyl-derivatives may be advantageously employed for the separation of cadaverine and putrescine (page 340).

Neuridine, $C_5H_{14}N_2$, is a diamine of unknown constitution, isomeric with cadaverine and saprine. It was discovered by Brieger in 1884, in the products of the putrefaction of the flesh of mammals, fish, cheese, and gelatin. It has also been found in various organs of the human body, among others in fresh brains.

Neuridine appears to be one of the most constant products of the putrefaction of albuminoid substances. Its formation has been observed in the putrefaction of yolk of egg, gelatin, fish, horse-flesh, &c. It is generally accompanied by choline, appearing about the second or third day, and being still recognisable until the fourteenth (compare page 325).

Brieger recommends the following method for the preparation of neuridine:—Finely chopped flesh is treated with some water, and the mixture allowed to putrefy for five or six days at a temperature of about 40° C. The liquid is then separated from the solid matters by pressure, boiled, and filtered. The filtrate is saturated, after cooling, with mercuric chloride, which precipitates the neuridine, while certain other ptomaïnes remain in solution. The precipitate is filtered off, washed, suspended in water, and decomposed by sulphuretted hydrogen. The liquid, filtered from the mercuric sulphide, is concentrated on the water-bath, until it deposits, on cooling, fine needles of neuridine hydrochloride, which may be purified by repeated crystallisation from hot dilute alcohol.

The following alternative method for the isolation of neuridine was employed by Brieger in his earlier researches. It is of interest from its general applicability to the isolation of analogous ptomaïnes, and from the light it affords of the behaviour of neuridine to solvents and reagents:—The liquid obtained by treating the putrefying animal matters with water is strained, boiled, filtered, precipitated with lead acetate, and the excess of lead removed by sulphuretted hydrogen. The liquid thus clarified is evaporated to a syrup and exhausted with amylic alcohol. The extract is treated several times with water and evaporated, then strongly acidulated with sulphuric acid, and agitated repeatedly with ether to extract hydroxy-acids of the aromatic series. It is next concentrated to one-fourth of its volume to evaporate volatile fatty acids. The sulphuric acid is then removed by baryta, the excess of baryta by carbon dioxide, and the liquid then heated for some time on the water-bath. After cooling, the liquid is saturated with mercuric chloride, the precipitate carefully washed and decomposed with sulphuretted hydrogen. On concentrating the liquid, crystals of inorganic compounds are first deposited. These are filtered off and washed with absolute alcohol. The filtrate and washings, when further concentrated, deposit large needles of neuridine hydrochloride.

Free neuridine is a gelatinous substance, having a strong odour resembling that of semen. It is very readily soluble in water, but is insoluble in absolute alcohol and in ether, and is not readily soluble in amylic alcohol. When boiled with caustic soda, it is decomposed with formation of di- and tri-methylamine.

When mixed with other matters of putrefactive origin, neuridine is said to possess poisonous characters allied to those of peptotoxine; but pure neuridine is stated by Brieger to be perfectly innocuous.

Neuridine hydrochloride, $C_5H_{14}N_2,2HCl$, crystallises in long needles extremely soluble in water and dilute alcohol, but in the pure state the salt is insoluble in absolute alcohol, amylic alcohol, ether, chloroform, benzene, or petroleum spirit. Its behaviour with solvents is gravely modified by the presence of other animal matters, and hence, in extracting acid liquids containing these by various immiscible solvents, the neuridine hydrochloride is dissolved more or less completely. This behaviour is the more important since neuridine appears to occur very generally in animal tissues, and not improbably plays a leading part in nutritive changes.

When heated, neuridine hydrochloride sublimes in needles, which have a red or blue colour owing to partial decomposition.

A solution of neuridine hydrochloride reacts with many of the general reagents for alkaloids. With picric acid it yields gradually a precipitate which rapidly assumes the form of fine yellow needles, which decompose without previous fusion at about 230° C. With phospho-tungstic acid it yields a white, amorphous precipitate, soluble in excess of the reagent; and with phospho-molybdic acid a white, crystalline precipitate. Potassio-iodide of bismuth produces a red, amorphous precipitate. B,H_2PtCl_6 crystallises in flat needles, and is precipitated from its aqueous solution by addition of alcohol. The *aurichloride* is said to contain $B,(HCl)_2,2AuCl_3$, which corresponds to 41·19 per cent. of gold. It crystallises in bunches of yellow needles, sparingly soluble in cold water. With mercuric chloride,[1] Mayer's reagent, potassio-iodide of cadmium, iodised potassium iodide, hydriodic acid, tannic acid, Fröhde's reagent, and a mixture of potassium ferricyanide and ferric chloride, neuridine gives negative reactions.

CADAVERINE, $C_5H_{14}N_2$, *i.e.*, $NH_2.(CH_2)_5.NH_2$, is isomeric with neuridine, but has the constitution of a pentamethylene-diamine. It has been produced synthetically, and is formed like neuridine in the bacterial decomposition of albuminous matters, but makes its appearance at a later stage of the putrefaction. It has been found in the urine and fæces in cases of cystinuria, and has been isolated (by the benzoyl chloride method) from the fæces of a person suffering from tertian ague.

Cadaverine may be isolated by the same means as neuridine, but is extremely difficult to obtain pure. The pure base is described as a clear, syrupy liquid, having an odour at once resembling that of mice and of semen, whence it has been called "cadaveric conine." When dehydrated by contact with caustic potash, cadaverine boils between 115° and 120° C. It volatilises with steam, and may be distilled unchanged in presence of caustic alkalies.

Cadaverine is not poisonous. It is a strong base, absorbing carbon dioxide from the air to form a crystalline carbonate.

Cadaverine hydrochloride, $B(HCl)_2$, forms deliquescent needles, or from alcohol in fine plates. It is insoluble in perfectly absolute alcohol, but dissolves readily in spirit of 96 per cent., whereas the putrescine salt dissolves with difficulty. When heated, cadaverine hydrochloride decomposes into piperidine and ammonium chloride:—

$$CH_2:\left\{\begin{array}{l} CH_2.CH_2.NH_2 \\ CH_2.CH_2.NH_2 \end{array}\right. = NH_3 + CH_2:\left\{\begin{array}{l} CH_2.CH_2 \\ CH_2.CH_2 \end{array}\right\}:NH$$

Cadaverine aurichloride is said to contain $B,2(HAuCl_3)$, corresponding to 50·41 per cent. of gold. It crystallises sometimes in long brilliant needles and sometimes in deliquescent cubes, melts at

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[1] Free neuridine is stated to yield a precipitate with mercuric chloride, and is also said to be precipitated by neutral and basic lead acetates.

188°, and is readily soluble in water. B,H_2PtCl_6 forms dirty red needles, but by repeated recrystallisation is obtained in bright yellow orthorhombic prisms. It is decomposed at about 235°, and is insoluble in cold water, but dissolves with difficulty on heating.

The *compound*, B,$(HCl)_2$,$4HgCl_2$ ($Hg = 63{\cdot}54$ per cent.), melts at 214° to 216°, and is insoluble in alcohol and in cold water, whereas the corresponding salt of putrescine is soluble in cold water. The *salt*, B$(HCl)_2$,$3HgCl_2$, has also been described, the composition of the compound obtained depending on the quantity of mercuric chloride employed.

Cadaverine oxalate, B,$H_2C_2O_4 + 2H_2O$, is obtained by treating the free base with an alcoholic solution of oxalic acid. It crystallises in needles which melt at 160° with evolution of gas, and are insoluble in absolute alcohol. With excess of oxalic acid cadaverine yields the salt B,$(C_2H_2O_4)_2 + H_2O$, which crystallises in quadratic plates insoluble in absolute alcohol and melting with decomposition at 143° C.

Cadaverine picrate, B,$(C_6H_3N_3O_7)_2$, is soluble with difficulty in cold water and insoluble in absolute alcohol. It melts at 221°, with decomposition.

Dibenzoyl-cadaverine, $C_5H_{10}(NH.Bz)_2$, is a compound of interest as affording a means of isolating cadaverine and separating it from its homologues. For this purpose, the liquid (urine, or clarified extract of the putrefying substance) is treated with half its measure of a 10 per cent. solution of caustic soda, and some (5 per cent.) benzoyl chloride. The mixture is shaken at intervals until the odour of benzoyl chloride is no longer perceptible, when it is filtered, and the filtrate treated with a further quantity of benzoyl chloride, to remove any diamines which had previously escaped precipitation. The precipitate is dissolved in alcohol, and the solution poured into a relatively large quantity of water, when the dibenzoyl compounds will be gradually deposited in a crystalline form. To separate the cadaverine and putrescine compounds, the crystalline precipitate is filtered off, washed with cold water, and dissolved in warm alcohol. The solution is poured into twenty times its measure of ether, when crystals of putrescine-dibenzoyl will gradually separate, whereas the corresponding compound of cadaverine is more soluble, and may be recovered on evaporating the solution.

Dibenzoyl-cadaverine crystallises in long needles and plates which melt at 130°. It is readily soluble in alcohol, moderately soluble in ether, and insoluble in water. It is unaffected by dilute acids and alkalies, but when dissolved in alcohol and boiled for a long time with strong hydrochloric acid, it is gradually decomposed into benzoic acid and cadaverine. After removing the alcohol by evaporation, the benzoic acid can be extracted by agitating with ether, and the cadaverine then precipitated as chloroplatinate.

Cadaverine is precipitated by most of the general reagents for alkaloids. Ferric ferricyanide is only slowly reduced to prussian blue by a solution of cadaverine hydrochloride.

When heated with chloroform and alcoholic potash, cadaverine does not yield an isonitrile (compare Part ii. page 7). Treated in alcoholic solution with excess of methyl iodide it yields a compound probably containing $C_5H_{14}N_2.2MeI$.

PUTRESCINE, $C_4H_{12}N_2$, is a ptomaïne which usually accompanies cadaverine in putrefactive decomposition, but makes its appearance at a somewhat later stage. It is sometimes described as tetramethylene-diamine, but its true constitution appears to be that of a dimethyl-ethylene-diamine:—$C_2H_4\left\{\begin{matrix}NH.CH_3\\NH.CH_3\end{matrix}\right.$.

Putrescine has been obtained synthetically by the reducing action of sodium on ethylene cyanide in alcoholic solution.

Putrescine has been found in the urine and fæces in cases of cystinuria, its isolation being effected as described under cadaverine.

Putrescine is a liquid having a peculiar spermatic odour.[1] It boils at 135°, and is not poisonous.[2] It absorbs carbon dioxide from the air, and forms crystallisable salts. $B,(HCl)_2$ forms long colourless needles, readily soluble in water, but insoluble in absolute alcohol. B,H_2PtCl_6 forms hexagonal plates, soluble with difficulty in cold water. $B,(HAuCl_4)_2+2H_2O$ is insoluble in cold water, whereas cadaverine aurichloride is readily soluble. Putrescine picrate crystallises in difficultly soluble yellow plates, which melt with decomposition at about 250°.

Dibenzoyl-putrescine, $C_4H_8(NH.Bz)_2$, forms long needles or silky plates melting at 175° to 176°, and is less soluble in alcohol or ether than the corresponding cadaverine compound (page 340).

SAPRINE, $C_5H_{14}N_2$, isomeric with cadaverine and neuridine, was found by Brieger in human liver and spleen after putrefaction had continued for several weeks. Saprine closely resembles cadaverine, but presents the following differences :—Cadaverine chloroplatinate is but very slightly soluble, and crystallises in rhombs; while the platinum salt of saprine is readily soluble and forms groups of parallel needles. Saprine does not form a definite aurichloride, while its hydrochloride crystallises in flattened needles which are permanent in the air, the corresponding salt of cadaverine being very deliquescent. Free saprine reduces ferric ferricyanide to prussian blue. It is precipitated by mercuric chloride. Saprine is not poisonous.

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[1] Putrescine has also been described as crystallising in scales melting at 24° and boiling at 156° to 157°. Possibly this description applies to a preparation which had been exposed to the air and consisted largely of putrescine carbonate.

[2] On the other hand, the tetramethyl-derivative of putrescine, $C_4H_8Me_4N_2$, obtained by repeated treatment of the base with methyl iodide, occasions poisonous symptoms similar to those produced by muscarine.

Gerontine, $C_5H_{14}N_2$, a ptomaïne isomeric with cadaverine and saprine, was extracted by V. Grandis (*Jour. Chem. Soc.*, lx. 587) from the cell-nuclei of the liver and some other organs of dogs, in which it occurs as crystals of the phosphate (compare spermine, page 200). Gerontine is a strongly basic, yellowish liquid of disgusting odour, which is soluble in water and gradually crystallises from its solution in spherical tufts. The hydrochloride, chloroplatinate, mercurochloride, picrate, &c., are crystallisable.

Mydaleine is a very poisonous ptomaïne isolated by Brieger from putrefying animal matters. It is probably a diamine, but the quantity obtained was insufficient to establish its constitution and formula with certainty. Mydaleine forms a very soluble chloroplatinate (containing Pt, 38·74; C, 10·83; and H, 3·25 per cent.) obtainable in crystals from its solution in absolute alcohol.

When injected hypodermically, mydaleine occasioned in guinea-pigs dilation of the pupil, increased temperature, and abundant secretion from the nose and eyes.

According to Brieger, when mydaleine is administered to rabbits the secretion from the lachrymal glands and nostrils is increased, the pupils are dilated and reactionless, the temperature increased. The pulse and respiration are at first more rapid, but subsequently become slower, the heart finally arresting in diastole. Increased peristalsis, diarrhœa, vomiting, clonic spasms, and paralysis, with a tendency to stupor, precede death. Five grains of mydaleine hydrochloride administered hypodermically to a kitten caused rapid dilation of the pupils, diarrhœa, vomiting, salivation, stupor, muscular spasms, paralysis first of the hind and then of the fore legs, slow laboured breathing, and death, the heart being arrested in systole.

Phlogosine, a ptomaïne obtained by Lebur from pure cultivations of *Staphylococcus aureus*, probably belongs to the diamine class.

Spasmotoxine is also probably a diamine. It was isolated by

Brieger from pure cultivations of the tetanus bacillus, and produced tonic and clonic convulsions. An accompanying unnamed *base* caused tetanus, accompanied by a flow of saliva and tears.

Unclassified Ptomaïnes.

In addition to the ptomaïnes of the diamine class and others mentioned in the table on pages 332, 333, the following have been described:—

BASES isomeric with collidine and coridine, and having the formula $C_8H_{11}N$ and $C_{10}H_{15}N$ respectively, have been isolated by O. de Coninck from the muscular tissues of the cuttle-fish after bacterial putrefaction (*Comp. rend.*, cvi. 858; cx. 1339; cxii. 584; abst. *Jour. Chem. Soc.*, liv. 730; lviii. 1170; lx. 845).

A BASE, to which the improbable formula $C_7H_{38}N_4$ is ascribed, was obtained by Gautier from putrefied meat.

TETANOTOXINE, $C_5H_{11}N$, is a ptomaïne extracted by Brieger from cultivations of the tetanus bacillus, together with tetanine, $C_{13}H_{30}N_2O_4$, and spasmotoxine, a ptomaïne of unknown composition. Tetanotoxine is described as a colourless liquid of disagreeable odour, capable of distillation either alone or with steam. B,HCl melts at about 130°, and dissolves in water and in alcohol. B,H_2PtCl_6 is difficultly soluble and decomposes at 240°, while $B,HAuCl_4$ melts at 130° and is readily soluble. Injected subcutaneously, it produces tremor and paralysis, followed by violent convulsions.

The formulæ attributed to the following five ptomaïnes render it probable that they belong to the betaïne class.

MYDATOXINE, $C_6H_{13}NO_2$, was isolated by Brieger by the mercuric chloride process from putrefying corpses and horse-flesh. It is a syrup of strongly alkaline character, which in large doses produces symptoms resembling those due to curare. Lachrymation, diarrhœa, and convulsions have been recorded. B,HCl is deliquescent, and yields with phospho-molybdic acid a compound crystallising in cubes. B,H_2PtCl_6 melts at 193°, and is very soluble in water.

A non-poisonous *base* of the same formula as mydatoxine has been obtained by Brieger from pure cultivations of the tetanus bacillus.

MYTILOTOXINE, $C_6H_{15}NO_2$, is a ptomaïne separated by Brieger from decomposing mussels, and is believed to be the active agent in mussel-poisoning.[1]

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[1] For the isolation of mytilotoxine, Brieger boiled the mussels with water acidulated with hydrochloric acid, evaporated the filtered liquid to a syrup, and exhausted the residue *thoroughly* with alcohol. The filtered liquid was

Mytilotoxine is a base of very peculiar and characteristic odour, but both the odour and the poisonous effects decrease and ultimately disappear by exposure to air. The base is readily decomposed by boiling with sodium carbonate, but in acid solution (*e.g.* the hydrochloride) mytilotoxine may be boiled and evaporated to dryness without change. B,HCl forms tetrahedral crystals B,$HAuCl_4$ crystallises in cubes and melts at 182° C.

By extracting poisonous mussels with hot alcohol, E. Salkowski (*Chem. Centr.*, 1886, 24) obtained a highly poisonous substance which was not precipitated by platinum chloride, nor volatile with steam, but which was decomposed on boiling with sodium carbonate. A solution containing 0·0055 of dry substance was sufficient to kill a rabbit. The poisonous mussels yielded a green alcoholic extract, while non-poisonous mussels give a nearly colourless extract; from which Salkowski infers that the poison is probably contained in the liver of the animal.

MYDINE, $C_8H_{11}NO$, found by Brieger in putrefying corpses, has a strongly ammoniacal odour. It is not poisonous, is a powerful reducing agent, precipitating metallic gold from auric chloride, and forms a picrate crystallising in large prisms melting at 195°.

GADININE, $C_7H_{17}NO_2$, is a ptomaïne found by Brieger in putrefied herrings and cod. When the muscarine is precipitated as chloroplatinate, the gadinine remains in solution, but on cautious evaporation the mother-liquor deposits the platinum salt in golden-yellow plates, sparingly soluble in water. When the chloroplatinate is decomposed by sulphuretted hydrogen, and the filtrate evaporated, the *hydrochloride* is obtained in large colourless needles, very soluble in water but insoluble in alcohol. Gadinine chloroplatinate has been prepared, but the base does not appear to form an aurichloride. It is not poisonous.

TYPHOTOXINE, $C_7H_{17}NO_2$, produced by the typhus bacillus, has been described by Brieger. It is a strong base of alkaline reaction, and possesses poisonous properties. B,$HAuCl_4$ forms

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treated with alcoholic lead acetate, the filtrate evaporated, and the residue again extracted with alcohol. From the solution the lead was removed by sulphuretted hydrogen, the alcohol evaporated, water added to the residue, and the liquid decolorised by animal charcoal. The free acid was then neutralised by sodium carbonate, acidulated with nitric acid, and treated with phospho-molybdic acid. The precipitate was decomposed by warming with a solution of neutral lead acetate, the lead removed from the filtrate by sulphuretted hydrogen, the liquid acidulated with hydrochloric acid, and evaporated to dryness. On treatment with absolute alcohol, any betaine chloride, &c., remained undissolved, while the pytilotoxine was precipitated from the filtrate by an alcoholic solution of mercuric chloride.

elting at 176°. The hydrochloride and phospho-tungstate
allisable. Typhotoxine is believed to be the chemical
typhoid fever. An isomeric *base* was found by Brieger
flesh which had been putrefying four months. It was
s, formed no picrate, but gave a chloroplatinate. $B,HAuCl_4$
eedles or plates melting at 176° and sparingly soluble in

INE, $C_{13}H_{30}N_2O_4$, was obtained by Brieger by inoculat-
beef-broth with the tetanus bacillus, and found also in
orpses. It is a strongly alkaline yellow syrup, permanent
e solutions but readily decomposing in presence of acids.
very deliquescent. B,H_2PtCl_6 is soluble in water and in
but is precipitated from its solution in the latter solvent
g ether. It crystallises in plates which decompose at
7°. Tetanine is very poisonous, producing tetanic con-
and death.[1]

MAÏNE containing $C_{16}H_{24}N_2O_4$ has been isolated by C.
e (*Compt. rend.*, cxviii. 476) from a ripe cheese from
k, which gave rise to digestive troubles when eaten. From
mme of the cheese some decigrammes of a well crystallised
e extracted by Gautier's process. It was odourless, bitter,
htly soluble in water, but soluble in alcohol. The
solution had a specific rotation of +11·3°. The hydro-
was very soluble and crystallised in large needles, and
num and gold salts were also crystallisable. The solutions
cipitated by picric acid, phospho-molybdic acid, iodised
iodide, but not by tannin. Introduced into the stomach

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ne was isolated from beef-broth cultures of the tetanus bacillus by
o and Weyl in the following manner:—The broth was digested for
s at 40° C. with water acidulated with hydrochloric acid (0·25 per
was then made feebly alkaline and distilled *in vacuo*. The distil-
ined tetanotoxine, ammonia, indol, &c. The liquid left in the
ained the tetanine, and was treated by Brieger's process with mer-
ride and filtered. The filtrate, which contained most of the
was freed from mercury by sulphuretted hydrogen, evaporated, and
exhausted with absolute alcohol. The filtered liquid was treated
lic platinic chloride, which precipitated ammonia and creatinine,
e tetanine. On filtering and adding ether to the filtrate, the
alt of tetanine was precipitated. The salt thus obtained was very
t, hence it was filtered off rapidly and dried *in vacuo*. When
ed from hot 96 per cent. spirit, the platinum salt formed clear
tes, which after drying in a vacuum were only with difficulty
water. By decomposing the chloroplatinate by sulphuretted
and treating the resultant hydrochloride with freshly-precipitated
ver, a solution of the free base was obtained.

of a pig the base produced diarrhœa; but an aqueous solution of the hydrochloride injected into the vein of a rabbit's ear produced no ill effects. Other cheeses of the same kind yielded no similar base.

A BASE containing $C_{14}H_{20}N_2O_4$, or $C_7H_{10}NO_2$, possibly identical with tyroleucine, was obtained by Guareschi from putrefied fibrin. It crystallises from alcohol in brilliant plates, melting at 248° to 250°, and is soluble in water and alcohol, but very sparingly in chloroform.

BASES represented by the formulæ $C_5H_{12}N_2O_4$ and $C_7H_{12}N_2O_6$ were extracted by G. Pouchet from putrid animal substances. They form crystallisable hydrochlorides and chloroplatinates, and are very poisonous.

SARDININE, $C_{11}H_{11}NO_2$, was extracted by A. B. Griffiths from the products of the bacterial fermentation of sardines, by the methods of Gautier and Brieger. It is described as white, crystalline, feebly alkaline in reaction, and soluble in water. The hydrochloride, aurichloride, and chloroplatinate are crystallisable. Sardinine gives a greenish precipitate with phospho-molybdic acid, yellowish-white with phospho-tungstic acid, and yellow with picric acid. It is precipitated by Nessler's reagent and by silver nitrate. Sardinine is poisonous, producing vomiting, profuse diarrhœa, and death. It is probably the cause of the poisonous symptoms produced by decomposing or badly-preserved sardines.[1]

PTOMAÏNES having characteristic properties have been extracted by A. P. Luff (*Brit. Med. Jour.*, 1889, page 193) from the urine of patients suffering from scarlet and typhoid fevers,[1] by the following process, which presupposes the bases to be soluble in ether :—

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[1] "Some persons exhibit an idiosyncrasy to being poisoned by fish, while to others no harm seems to happen under any circumstances. Arnstamoff has observed 11 cases of poisoning in human beings after eating salted salmon; of these 5 died. An examination of the fish showed a peculiar soft consistency, but no putrefaction. A large number of living micro-organisms were seen under the microscope, and these bore a strong resemblance to typhoid bacilli. Symptoms of poisoning developed in the patients in ten to twenty-eight hours after ingestion of the fish, but the amount ingested had no influence on the rapidity and intensity of the toxic symptoms. Complaint was chiefly made by the patients of general weakness, abdominal pains, dyspnœa, mydriasis, diplopia, vertigo, dryness in the mouth, dysphagia, constipation, and a lowered temperature. The *post-mortem* signs in the fatal cases were very indefinite, and if anything only pointed to death from asphyxia. Bacteriological and microscopical examination of the various organs afterwards revealed the presence of the same microbes which had been detected in the fish. Pure cultures made with these microbes were injected into 19 rabbits, 2 dogs, and 2 cats. The latter four animals recovered, but only after severe illnesses, while all the rabbits succumbed. Both in the symptoms presented during life, and in the presence of the microbes in the organs after death, the toxic effects observed in the animals were identical to those noticed in the patients above referred to."—*Medical Press.*

A large quantity (several gallons) of the urine was rendered alkaline by sodium carbonate, and thoroughly shaken with half its measure of ether. After standing, the ethereal layer was separated and agitated with a solution of tartaric acid, which, after separation from the ether, was rendered alkaline with sodium carbonate, and the liquid again shaken with half its measure of ether. After standing, the ether was separated and allowed to evaporate spontaneously, the residue obtained being subsequently dried over concentrated sulphuric acid. When normal urine is subjected to this process, no alkaloidal residue is obtained, provided the patient has not been taking any alkaloidal or antipyretic remedy. From typhoid urine, Luff isolated a white, crystalline substance of alkaloidal character. From the urine of scarlet fever patients the base isolated was white, semicrystalline, and soluble in water with faintly alkaline reaction.

When dissolved in very dilute hydrochloric acid, Luff's fever bases gave the following reactions :—

Reagent.	*Typhoid Fever Base.*	*Scarlet Fever Base.*
Phospho-molybdic acid, . . .	White ppte.	Yellowish-white ppte.
Phospho-tungstic acid, . . .	No reaction.	White ppte.
Tannic acid, ...	Yellow-brown ppte.	No reaction.
Picric acid, ...	Dense yellow ppte.	Yellow ppte.
Platinic chloride, ...	No reaction.	No reaction.
Auric chloride, ...	Dense yellow ppte.	Slight yellow ppte.
Mayer's solution, ...	Dense yellow ppte.	Yellowish-white ppte.
Iodine solution, ...	Brown ppte.	Brown ppte.

Tyrotoxicon is the name given by V. C. Vaughan to a poisonous ptomaïne found by him in stale milk, ice-creams, and cheese. It is described as crystallising in needles, which gradually decompose on exposure to moist air. It has a "dry" taste, and an odour like that of stale cheese. Tyrotoxicon is soluble in water, alcohol, and chloroform. When pure, it is insoluble in ether, but dissolves in presence of impurities.

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[1] A. B. Griffiths has described various ptomaïnes and leucomaïnes as occurring in the urine of patients suffering from various diseases. His researches are to be found chiefly in *Comptes rendus* and the *Proceedings of the Royal Society of Edinburgh.*

Tyrotoxicon was found by Vaughan to act as a violent poison both on man and the lower animals. A minute portion placed on the tongue of a child produced sickness and diarrhœa, symptoms identical with those of *cholera infantum*. Ten drops of a solution of tyrotoxicon obtained from milk three months old, when given to a young dog, caused frothing at the mouth, vomiting, diarrhœa, and muscular spasms. Similar symptoms were obtained with cats. There was no inflammation of the stomach, the mucous membrane after death being white and soft.

If a strong alcoholic solution of tyrotoxicon be evaporated on the water-bath with some platinic chloride, a violent explosion occurs immediately the whole of the alcohol has evaporated.

Tyrotoxicon is not precipitated by the majority of the general reagents for alkaloids.

Tyrotoxicon forms a potassium derivative crystallising in six-sided plates, soluble in absolute alcohol, to form a solution precipitated by ether. Vaughan believes this compound to be diazobenzene-potassoxide, $C_6H_5.N_2.OK$, and considers tyrotoxicon itself to be identical with, or closely allied to, diazobenzene butyrate, in support of which view he advances the following arguments:—

a. Synthetically prepared diazobenzene butyrate forms crystals exactly similar to those of tyrotoxicon, and undergoes a similar decomposition in presence of aqueous vapour.

b. The platinum salts of diazobenzene and tyrotoxicon both explode violently when heated to a temperature approaching 100° C.

c. The coloration (yellow to orange-red) yielded by tyrotoxicon extracted from milk or cheese when treated with phenol and sulphuric acid is identical with that obtained when fresh milk is treated with synthetical diazobenzene nitrate, while a green colour is produced when the purified potassium compound of tyrotoxicon or diazobenzene is treated with the same reagent.

d. The gold salts of tyrotoxicon and diazobenzene agree in being insoluble in water but soluble in hot alcohol, crystallising from this solvent in golden-yellow plates, which are decomposed by repeated treatment with hot alcohol.

e. The proportion of potassium contained in the potassium derivative of tyrotoxicon agrees very closely (23·93 per cent. against 24·42 per cent.) with that present in the diazobenzene derivative of the formula $C_6H_5.N_2.OK$.

f. The poisonous effects of diazobenzene on the lower animals are identical with those produced by tyrotoxicon, and the *post-mortem* appearances of the stomach are similar.

The identity of tyrotoxicon with diazobenzene does not appear to be sufficiently established.

For the detection of tyrotoxicon in *milk* and *cheese*,[1] V. C. Vaughan recommends the following process (*Analyst*, xiii. 14):—The filtrate from the curdled milk, or the filtered cold-water extract of cheese, is neutralised with sodium carbonate, transferred to a separator, and shaken with its own volume of pure ether.[2] The mixture is allowed to stand for twenty-four hours, or until separation is effected, when the ethereal layer is allowed to evaporate spontaneously in an open dish. The residue is dissolved n water, the liquid again shaken with ether, and the ethereal ayer separated and allowed to evaporate as before. Repeated extractions with ether should be avoided, as the purer the tyrotoxicon becomes the less readily is it dissolved. The residue is next taken up in a few drops of distilled water, and the solution tested in the following manner:—

a. A drop of the liquid is treated on porcelain with a few drops of a recently-prepared mixture of equal volumes of phenol and concentrated sulphuric acid, free from nitrous compounds. In presence of tyrotoxicon, a coloration varying from yellow to orange-red, and ultimately becoming violet, will be obtained.

b. Treat the remainder of the solution with a concentrated solution of caustic potash, and evaporate to dryness on the water-bath. In presence of tyrotoxicon, diazobenzene-potassoxide will be formed, recognisable by its crystalline form and the green coloration which it gives on treatment with a mixture of phenol and strong sulphuric acid.

c. An acid solution of tyrotoxicon prepared from milk gives a golden-yellow precipitate with auric chloride, but the formation of the gold salt from some milks is extremely slow, apparently depending on the condition of the other organic matter present.

Vaughan recommends that the foregoing tests should be supplemented by extracting a further quantity of the milk or cheese, and administering an aqueous extract of the ethereal residue to a cat. He regards the physiological test as the best and most reliable.

Vaughan (*Chem. News*, lvi. 45, 52) examined twelve samples of suspected cheese by extracting it with alcohol and evaporating the extract *in vacuo* at a low temperature. The residue thus obtained produced poisonous effects on himself. He obtained needles of tyrotoxicon by rendering the liquid slightly alkaline with caustic soda and extracting with ether. On spontaneous evaporation, the ethereal layer gave a residue which reduced iodic acid and yielded prussian blue when treated with a mixture of ferric chloride with potassium ferricyanide. Vaughan does not regard these reactions as characteristic of tyrotoxicon, as peptones and other bodies existent in milk react similarly.

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[1] As tyrotoxicon decomposes with great facility, the milk or cheese should be exposed to the air as little as possible.

[2] Vaughan states that ordinary ether often contains an irritating ptomaïne-ike body.

H. A. Weber (*Jour. Amer. Chem. Soc.*, xii. 485 ; *Jour. Soc. Chem. Ind.*, x. 728) has examined numerous samples of poisonous cheese, and found that in all cases the aqueous solution of the ether-residue exerted a reducing action on ferric ferricyanide, and gave a reddish-yellow colour with a mixture of phenol and sulphuric acid, though it exhibited no marked poisonous effects. He considers that these reactions do not prove the presence of tyrotoxicon, but depend on the presence of an organic base (probably an amine), which reduces the ferric ferricyanide, and on that of butyric acid, which gives the coloration with phenol. He points out that both these bodies would be probable constituents of decomposing cheese and milk, and would probably be extracted by ether together in the form of a salt. He attributes to the presence of these bodies the orange-red coloration produced when diazobenzene is dissolved in whey, the liquid extracted with ether, and the residue treated with phenol and sulphuric acid.

Vaughan has found that three months are required for the formation of tyrotoxicon in milk kept in tightly-stoppered bottles, but its formation is greatly hastened by the addition to normal milk of some butyric acid ferment (such as is used in the preparation of butyric acid), the poison being then produced in about eight to ten days (*Analyst*, xv. 14). He considers that the poison is directly or indirectly the product of some organism, as in all samples of milk in which he detected poison he found a varying proportion of butyric acid, which points to the poison being the result of a butyric fermentation.[1] This conclusion is confirmed by the observation of R. H. Frith (*Pharm. Jour.*, [3], xviii. 92), who extracted from milk a ptomaïne-like body he called lactotoxine. He found that normal milk had to be kept about ten weeks before

---

[1] Some years ago, Selmi obtained a ptomaïne resembling conine, and pointed out that it might be a product of the action of butyric aldehyde or butyric acid on ammonia :—

$$2C_4H_8O + NH_3 = C_8H_{15}N + 2H_2O$$
$$2C_4H_8O_2 + NH_3 + 2H_2 = C_8H_{15}N + 4H_2O.$$

he poison could be detected, but that if a piece of rancid butter were suspended in the liquid the poison appeared in as little as our days. Lactotoxine produces poisonous symptoms similar to hose of tyrotoxicon, with which substance it is probably identical.

Sucholotoxine and **Saplagotoxine** are ptomaïnes extracted by on Schweinitz from pure cultivations of the microbe of hog-holera. Both are very poisonous.

By cultivating the bacillus of swine-fever in peptone-broth at 37°, von Schweinitz obtained a primary amine of unknown omposition, and a base the chloroplatinate of which contained $C_{14}H_{34}N_2PtCl_6$. B,HCl formed a syrup soluble in alcohol, which was not poisonous. The free base could not be isolated (*Chem. Centr.*, 1890, ii. 759 ; and *Jour. Chem. Soc.*, lx. 476).

Susotoxine, $C_{10}H_{26}N_2$, and an analogous ptomaïne were isolated by F. G. Novy (*Med. News*, Sept. 1890) from a cultivation of Salmon's bacillus in pork-broth. By Brieger's process with mer-uric chloride an amorphous precipitate was obtained, which, on reatment by the usual process for preparation of a chloroplatinate, yielded a remarkable compound, to which the formula $C_8H_{14}N_4PtO_8$ was ascribed. From the mother-liquor crystals of the platinum alt of susotoxine were obtained having the composition B,H_2PtCl_6. Susotoxine is described as being very poisonous and giving reactions with the general reagents for alkaloids.

Scombrine, $C_{17}H_{38}N_3$, is a ptomaïne obtained by Gautier and Etard from putrefied mackerel. It has an odour resembling yringa, and suffers decomposition at 100°. The chloroplatinate s crystallisable and soluble in water.

Morrhuine, $C_{19}H_{27}N_3$, was isolated by Gautier and Mourgues rom cod-liver oil. It is described as a yellowish liquid, very lkaline and caustic, having an odour of syringa. It possesses poisonous properties.

Aselline, $C_{25}H_{32}N_4$, accompanies morrhuine in cod-liver oil.[1]

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[1] In cod-liver oil, Gautier and Mourgues found butylamine, amyl-mine, hexylamine, and dihydrolutidine, in addition to aselline and morrhuine. According to J. Bouillot (*Compt. rend.*, cxvi. 439), the alkaloids of cod-iver are of biliary origin, and pre-exist in normal hepatic tissue ; and hence do ot belong to the class of ptomaïnes. He states that if a thin section of fresh od's liver be exposed to the vapours of hydrofluoric or hydrochloric acid for alf an hour, and then dried under a bell-glass for several hours, numerous rystals are observable under the microscope, among which may be recognised he hydrochlorides of morrhuine, aselline, and dihydrotoluidine. These rystals are never enclosed in the hepatic cellules, but are localised in the xtra-cellular fluid, and more especially about the periphery of the biliary ucts. If, after the above-named treatment, the section be moistened with

It is solid, odourless, insoluble in water, but soluble in alcohol and ether. It is poisonous in large doses.

ARSENICAL PTOMAÏNES. From the putrefying corpses of animals poisoned with arsenic Selmi extracted ptomaïnes which contained arsenic in a form not readily recognisable, and which gave precipitates with most of the alkaloidal reagents. T. Husemann (*Archiv. der Pharm.*, xvi. 415; *Chem. News*, xlv. 238) has suggested that the *Aqua Tofana* and *Acquetta di Perugia*, which played so important a part in Italian history some centuries since, owed their intense toxic action to the presence of certain ptomaïnes containing arsenic. According to tradition, the *Acquetta di Perugia* was prepared by rubbing white arsenic into the flesh of a recently-killed hog, and collecting the liquid which dropped therefrom in the course of putrefaction. Highly toxic arsines would not improbably be formed, in addition to which ammonium arsenite and other soluble compounds of arsenic would result, and hence a liquid far more poisonous than a simple aqueous solution of arsenious acid would be obtained.

Selmi has also shown that a volatile arsine is formed by the contact of arsenious oxide and albuminous matters, which possesses a strong toxic action differing somewhat from that of arsenious acid. Husemann has suggested that a similar compound may be formed from the size or paste used for fixing arsenical paper to the wall of a room, and may account for the poisonous effects experienced therefrom.

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platinic chloride, and again dried, barbed needle-shaped crystals of morrhuine chloroplatinate may be detected. Bouillot suggests the name pangaduine for the mixtures of bases obtained from cod-liver oil. It leaves 3·5 per cent. of ash on ignition, and dissolves in spirit, aqueous glycerin, &c. Pangaduine is said to be of value in tuberculosis, gout, rheumatism, diabetes, and other maladies characterised by imperfect nutrition. J. O. Schlotterbeek (*Pharm. Jour.*, [3], xxv. 585) extracted about 40 grammes of the slightly coloured salts of the mixed bases from 100 kilogrammes of light brown Norwegian cod-liver oil.

ANIMAL ACIDS.

acids occurring in the animal kingdom are in many instances
, oxalic, palmitic, benzoic) found also in plants, and a large
ortion of them have been prepared by artificial means. Hence
harp distinction can be drawn between bodies of acid function
d in animals and the organic acids from other sources ; just
o sharp distinction can be drawn between animal bases and
table alkaloids. A large number of the acids occurring in
animal kingdom have been already considered.[1] The acids
nging to the cyanogen-group will be described in the sequel.
re remain a limited number of bodies of acid function, which,
heir history and interest, are very closely associated with animal
nistry, and can be conveniently considered in this Chapter
er the heads of "Acids of Urine," "Acids of Bile," and
ctic Acids."

IDS OF URINE.

f the acids existing in urine, either in the free state or as salts,
ar the most important is uric acid (page 354). Hippuric
d (page 386) exists in the urine of the herbivora, and orni-
ric acid (page 386) in the urinary excrement of certain
s, while the urine of dogs has been found to contain kynure-
acid (page 355). Glycuronic acid is a urinary acid
onsiderable pathological interest. Urine also contains various
real sulphates, but the more important of these have already
described.[1] Pyrocatechol and other phenoloid bodies, some-
s occurring in urine, were described in Volume ii. ; while the
ler organic acids were considered in Volume i.[1]

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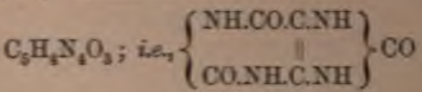
ormic, acetic, butyric, valeric, oxalic, and succinic acids were
ibed in Volume i. Palmitic, stearic, oleic, cerotic, and other of the
er fatty acids were considered in Volume ii. For phenyl-sulphuric,
l-sulphuric, benzoic and hippuric acids, see Vol. iii. Part i. Indoxyl-
huric and skatoxyl-sulphuric acids are described on page 302 *et seq.*, and
ric acid on page 358 of this Part.

Uric Acid. Lithic Acid.



$$C_5H_4N_4O_3;\ i.e., \left\{\begin{matrix} NH.CO.C.NH \\ \parallel \\ CO.NH.C.NH \end{matrix}\right\} CO$$

Uric acid is one of the most constant and characteristic products of tissue-metabolism in the animal organism. In its formation the nuclein of the cell-nuclei is specially concerned. Salts of uric acid are invariable constituents of human urine, the proportion present being greatly increased in cases of gout and rheumatism, when the urine contains large quantities of acid urate of sodium.[1] Traces of uric acid exist normally in the brain, lungs, liver, and spleen, and uric acid is also found in the saliva, gastric juice, sweat, &c. The merest trace exists normally in blood; but in cases of albuminuria, and especially of gout, the proportion becomes very appreciable. The so-called "chalk-stones," and other gouty concretions, commonly consist of the sparingly soluble acid sodium urate, while the buff-coloured sediment which frequently separates from human urine usually consists of the quadri-urate of sodium or ammonium. Acid urate of ammonium constitutes the greater part of the urinary excrement of birds ("guano"), while that of serpents and other terrestrial reptiles contains it in a still purer form. On the other hand, uric acid is nearly absent from the urine of herbivorous mammals, being replaced therein by hippuric acid.[2] In of dogs it is replaced by kynurenic and urocanic

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[1] The amount of uric acid commonly excreted by an adult is usually stated to be about 0·5 gramme (8 grains) in the twenty-four hours, but the results of the more modern methods of determination lead to the conclusion that the diurnal quantity eliminated under normal conditions is from 1·3 to 2·0 grammes (20 to 30 grains). The deposition of urates from urine on cooling does not prove their presence in the excretion in excessive amount.

According to K. Dapper (*Chem. Centr.*, 1895, i. 163) the absolute amount of uric acid excreted varies greatly in the same individual and in different people; while its relation to the total nitrogen of the urine varies from 23·2 to 122·4, according to the proportion of proteids in the food taken. He finds further no relationship between uric acid and body-weight; for 100 kilogrammes of the latter, the uric acid varies from 0·528 to 1·829 gramme.

During recent years, Haig and other observers have shown that uric acid has, in all probability, a much wider bearing in pathology than had previously been supposed. Its connection with gout, rheumatism, and stone has long been recognised, though its relation to these complaints has been much misunderstood. Its relation to other affections is even more obscure.

[2] F. Mittelbach (*Zeit. physiol. Chem.*, xii. 463; abst. *Jour. Chem. Soc.*, liv. 1215) has determined the uric acid in the urine of various herbivorous animals by Ludwig's method. The urine of cows and oxen contained from 0·008 to 0·045 gramme per 100 c.c., and uric acid was also present in the urine

nthesis of uric acid has been effected[2] by Horbac-

l horses. The urine of pigs contained from 0·003 to 0·030 gramme per 100 c.c.

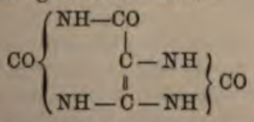
ENIC ACID. $C_{10}H_7NO_3$; *i.e.*, $C_9H_5(OH)N.COOH$. This substance stitution of a hydroxychinoline-carboxylic acid. The acid occurs in the urine of dogs, especially when fed on fat, but not normally rine. Kynurenic acid is best isolated by precipitating fresh dogs' phospho-tungstic acid in presence of hydrochloric acid, and liber-cid from the precipitate by treatment with baryta. Or the dogs' be concentrated to about one-third of its bulk, acidulated with ic acid, and allowed to stand for several days. On treating the with dilute ammonia, the kynurenic acid dissolves, leaving a ric acid.

ic acid crystallises with 2 aqua in long white needles, glittering al prisms, and other very characteristic forms. When heated to omes anhydrous, and melts at 204°. Kynurenic acid is practically n cold water, and requires about 110 parts of boiling water for It is soluble in boiling dilute hydrochloric or sulphuric acid, is oluble in hot alcohol, and readily in dilute ammonia. Kynurenic eble acid, but forms crystallisable salts. It dissolves readily in alkalies, and on boiling in sodium carbonate solution, lime-water, water. The calcium salt forms short, sparingly soluble, needles, d in stellate forms. The *barium* salt forms plumose groups of soluble nacreous needles, or more frequently curious triangular highly characteristic microscopic appearance. (Barium urate is uble even in boiling water.) Carbon dioxide precipitates free acid from a solution of the barium salt. From solutions of soluble s hydrochloric acid precipitates kynurenic acid, soluble in excess. n from uric acid.)

nurenic acid is evaporated at 100° with hydrochloric acid and a otassium chlorate, a reddish residue is obtained, which on addition becomes brownish-green and finally emerald-green. (Compare st, page 360.) Kynurenic acid gives a yellow coloration when it d with nitric acid, and the residue treated with ammonia.

ated to about 250°, kynurenic acid splits up into carbon dioxide oxychinoline or kynurine, $C_8H_6(OH)N$.

interesting synthesis of uric acid has been effected by Behrend n (*Berichte*, xxi. 999) by the reaction of aceto-acetic ether and this synthesis the following constitutional formula, first proposed us, has been assigned to uric acid:—



$$CO\left\{\begin{array}{l} NH-CO \\ \qquad\quad | \\ \qquad\quad C-NH \\ \qquad\quad \| \\ NH-C-NH \end{array}\right.\left.\begin{array}{l} \\ \\ \\ \\ \end{array}\right\}CO$$

ula shows that uric acid contains the residues of two molecules of zeweski by heating glycocine with ten times its weight of urea to about 230° C. :—

$$\underset{\text{Glycocine.}}{C_2H_5NO_2} + \underset{\text{Urea.}}{3CH_4N_2O} = \underset{\text{Uric acid.}}{C_5H_4N_4O_3} + \underset{\text{Water.}}{2H_2O} + \underset{\text{Ammonia.}}{3H_3N}.$$

Conversely, when uric acid is heated under pressure to 170° with hydriodic acid, it yields glycocine, ammonia, and carbon dioxide.

Uric acid differs by an atom of oxygen from xanthine, a feeble base of wide occurrence in both the animal and vegetable kingdoms, and the physiological and pathological relations of which are but little understood. (See page 305 *et seq.*)

Uric acid is best obtained by boiling serpents' excrement with dilute caustic alkali and treating the filtered liquid with excess of hydrochloric acid. On cooling, uric acid is deposited in a nearly pure state.[1] Or guano may be boiled with a solution of 1 part of borax in 120 of water, and the filtered liquid precipitated with hydrochloric acid. Or it may be first treated with dilute hydrochloric acid to remove the phosphates, the residue boiled with dilute caustic alkali, and the filtered liquid treated with excess of hydrochloric acid. Another convenient source of uric acid is the yellowish deposit of acid urates formed on urinals. This may be boiled with caustic soda as long as ammonia is evolved, carbon dioxide passed through the filtered liquid, and the precipitated acid urate of sodium washed with cold water, dissolved in caustic alkali, and the solution decomposed with acetic or hydrochloric acid. The product may be purified by dissolving it in hot caustic alkali, boiling with a little permanganate or bichromate of potassium, and filtering the liquid into excess of dilute hydrochloric acid. Uric acid may also be purified by solution in concentrated sulphuric acid and precipitation by addition of water.

When quite pure, uric acid forms a white crystalline powder without taste or smell, and of a specific gravity ranging from 1·855 to 1·893. On heating, it decomposes without melting,

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urea, and explains the fact that the decompositions of uric acid almost invariably yield either a molecule of urea or some derivative of urea, together with a second body which can by further treatment be converted into urea. Many of the decomposition-products of uric acid can indeed be prepared directly from urea. In view of the close relationship existing between urea and uric acid, it is not surprising that the foods which in the mammal cause an increased excretion of urea in birds are converted into uric acid.

[1] For the isolation of uric acid from birds' excrement, the substance should be boiled with milk of lime as long as ammonia is evolved. The liquid is filtered boiling hot, when a filtrate is obtained not more highly coloured than urine, whereas caustic soda or potash gives a highly coloured liquid. From the solution the uric acid is precipitated by hydrochloric acid.

hydrocyanic acid and carbon dioxide, yielding a sub-
taining cyanuric acid, ammonium cyanate and urea, and
carbonaceous residue.

lowly deposited from dilute solutions, uric acid separates
rystals containing $C_5H_4N_4O_3+2H_2O$. As obtained by
on of hydrochloric acid to cold filtered urine, uric acid is
anhydrous, but this statement is probably erroneous.
s Edmunds (in a communication to the author) states
ystals of uric acid thus obtained effloresce and break up
to 95° C., or even on exposure at the ordinary tem-
nd pressure for twenty-four hours over sulphuric acid.

ipitated by adding hydrochloric acid to urine, uric acid
ll crystalline scales, which are very apt to appropriate
matter.[1] The microscopic appearance of uric acid is very
When deposited from urine or other impure solutions,
, whetstone, and lozenge-like forms are among the most
nd characteristic (page 377, fig. 19 A, *b* and *c*). A. E.
as shown that the pigments of urine are especially con-
modifying the forms assumed by the uric acid, and that
ce of excess of one particular pigment will produce a cor-
definite variation in the form of the crystals. Dr J.
s has independently found that the forms assumed by
greatly depend on the nature and amount of the co-
ubstances. When precipitated from a solution of a pure
ddition of hydrochloric acid, uric acid generally forms
nsparent rhombic plates (page 377, fig. 19 A, *a*). Large
re obtainable much more readily from urine or other
lutions than from pure urates.

id is nearly insoluble in water, requiring 15,000 parts of
800 of boiling water for its solution. Blarez and
(*Compt. rend.*, civ., 1847) find that 100 grammes of
° C. dissolve 2·0 milligrammes of uric acid; at 10°, 3·7;
; and at 100°, 62·5 milligrammes of uric acid. Uric
lves in glycerin, but in alcohol and ether it is quite
Uric acid is soluble in solutions of the borates, phos-
bonates, acetates, and lactates of potassium and sodium,
solutions of the corresponding ammonium salts.

g sulphuric acid, uric acid dissolves to form a crystal-
phate, which is decomposed by water, the uric acid being

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d coloured by cruentin (hæmatoporphyrin) is pink, and usually
zor-shell shape. Urobilin is stated not to influence the colour or
uric acid crystals, and the shape is also unaffected by cruentin.
A. E. Garrod, in *Jour. Pathol. and Bacteriology* for Nov. 1895,

precipitated unchanged. When strongly heated with concentrated sulphuric acid, uric acid is broken up, the nitrogen being wholly converted into ammonia.

Hydrochloric acid has neither solvent nor chemical action on uric acid under ordinary circumstances; but when heated with concentrated hydrochloric or hydriodic acid under pressure to 170°, uric acid is decomposed with formation of glycocine, ammonia, and carbon dioxide.

By the action of oxidising agents on uric acid a number of compounds of great theoretical interest are obtainable. These form two distinct series. The compounds of the first class, represented by alloxan, $C_4H_2N_2O_4$, are produced by acid oxidising agents, such as nitric acid. Those of the second class, of which allantoïn, $C_4H_6N_4O_3$, is the type, result from the oxidation of uric acid in alkaline or neutral solution.

By treatment with strong nitric acid in the cold, uric acid yields alloxan and urea :—$C_5H_4N_4O_3 + H_2O + O = C_4H_2N_2O_4 + CO.(NH_2)_2$. Alloxan forms fine colourless crystals, very soluble in water and alcohol. The solution has an acid reaction, disagreeable astringent taste, and stains the skin red or purple after a time. Alloxan is decomposed by alkalies, and by oxidising and reducing agents. With ferrous sulphate, it gives a deep blue solution, precipitated on addition of an alkali. By further oxidation, alloxan is converted into parabanic acid, with evolution of carbon dioxide :—

$$CO\left\{\begin{array}{l}NH.CO\\NH.CO\end{array}\right\}CO + O = CO\left\{\begin{array}{l}NH.CO\\NH.CO\end{array}\right\} + CO_2$$

ALLOXAN (mesoxyl-urea). PARABANIC ACID (oxalyl-urea).

Parabanic acid is also produced by the direct treatment of uric with moderately strong and hot nitric acid. It forms colourless crystals, readily soluble in water to a strongly acid liquid. When heated with alkalies parabanic acid assimilates the elements of water and yields oxaluric acid.[1]

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[1] OXALURIC ACID, $NH_2.CO.NH.CO.COOH$, forms a white, crystallin powder of acid taste and reaction. It is but sparingly soluble in cold wat When boiled for some time with water or dilute alkali it splits into urea an oxalic acid :—

$$NH_2.CO.NH.CO.CO.OH + H.OH = NH_2.CO.NH_2 + OH.CO.CO.OH.$$

Ammonium oxalurate is stated to exist in small quantity in human urin from which it can be extracted by rendering a large volume (50 litres) of th liquid faintly alkaline to litmus, filtering from the resultant precipitat and passing the clear liquid through a moderate quantity of animal charcoa The charcoal is then washed with cold water till free from chlorides, dried at

Hot dilute nitric acid converts uric acid into alloxantin, $C_8H_4N_4O_7$, a body which is also produced by the action of reducing agents on alloxan. It forms colourless crystals, soluble with difficulty in cold water, but more readily at 100°. The solution reddens litmus, gives with baryta-water a violet precipitate, which turns white and disappears on heating, and reduces silver nitrate. Moistened with ammonia, or exposed to ammoniacal vapours, alloxantin yields a magnificent purple colour, due to the formation of ammonium purpurate or murexide, $(NH_4)C_8H_4N_5O_6$. The formation of this body furnishes a delicate and characteristic test for uric acid (see page 360).

Chlorine and bromine convert uric acid at ordinary temperatures into urea and alloxan. On heating, parabanic and oxalic acids are also produced. Hypobromites and hypochlorites cause the evolution of a portion of the nitrogen of uric acid in a gaseous state, but the reaction does not appear sufficiently definite to serve as a means of determining uric acid.

When oxidised in alkaline solution, by potassium permanganate or ferricyanide, uric acid yields carbon dioxide and allantoïn, $C_4H_6N_4O_3$.[1] By avoiding all rise of temperature, filtering, neutral-

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gentle heat, and boiled with alcohol. The alcoholic liquid is filtered, evaporated on the water-bath, the residue exhausted with tepid water, and the brownish liquid evaporated to a syrup. On standing in the cold, ammonium oxalurate gradually separates in crystals, which should be washed with absolute alcohol, and recrystallised from boiling water.

If a drop of a solution of pure ammonium oxalurate be allowed to evaporate, the salt appears under the microscope in the form of long pointed prisms, which reunite to form double hoops or rosettes. If the salt be impure, the hoops remain small and form globules armed with fine needles.

If a solution of ammonium oxalurate be treated with nitric acid, oxaluric acid crystallises out after a time dependent on the concentration. The crystals gradually disappear, and the liquid then contains urea nitrate, the characteristic crystalline forms of which can be observed by evaporating a drop and examining the residue under the microscope.

A moderately concentrated solution of ammonium oxalurate gives no precipitate with calcium chloride and ammonia, but on heating the liquid it becomes turbid even before the boiling-point is reached, and ultimately an abundant precipitate of calcium oxalate is formed.

Ammonium oxalurate gives no immediate precipitate with silver nitrate, but after a time silver oxalurate separates in fine needles, which do not blacken in the light, and dissolve in ammonia to a solution not reduced by boiling.

[1] ALLANTOÏN. $\mathrm{CO}\left\{\begin{array}{l}\mathrm{NH.CO}\\ \mathrm{NH.CH.NH.C.NH_2}\end{array}\right.$

Allantoïn is the diureïde of glyoxylic acid. It is the characteristic constituent of the allantoic fluid, especially that of the calf, is found in fœtal ising with acetic acid, and allowing the liquid to stand for twenty-four hours, crystals of allantoïn are obtained in nearly theoretical proportion.

When suspended in pure water, uric acid remains unchanged for a long time, but the addition of a very small quantity of fermenting urine causes its rapid and complete decomposition in hot weather, with formation of ammonium carbonate and other compounds.

DETECTION AND DETERMINATION OF URIC ACID.

Uric acid is commonly separated in the free state by adding excess of hydrochloric acid to its solution. When separated from urine in this manner, it forms a coloured deposit which adheres to the sides of the glass.[1] The best mode of operating is described in the sequel.

When isolated, uric acid is readily identified by its microscopic appearance, though the forms it assumes are very numerous (see page 377).

A highly characteristic and delicate reaction of uric acid is that known as the "murexide test," which is based on the behaviour of uric acid on oxidation. If uric acid, a urate, or even urine be treated with a few drops of strong nitric acid, and the liquid evaporated to dryness in porcelain at 100°, a yellowish or red

urine and amniotic fluid, and occurs in the urine of many animals shortly after birth, disappearing at a later period. It appears in the urine after internal administration of uric acid, and has been found in the young leaves of the plane-tree. Allantoïn forms shining colourless prisms of characteristic microscopic appearance (fig. 18). It is soluble in 160 parts of cold water, more readily in hot water or hot alcohol, but is insoluble in cold alcohol or in ether. Allantoïn has a neutral reaction, combines with metallic oxides, and is soluble in solutions of alkaline carbonates. It reduces Fehling's solution on prolonged boiling, and is precipitated by the nitrates of silver and mercury, which facts may be employed for its isolation. With furfurol and hydrochloric acid, it behaves like urea, but the coloration is less intense and less readily obtained. Allantoïn is best characterised by its crystalline form.

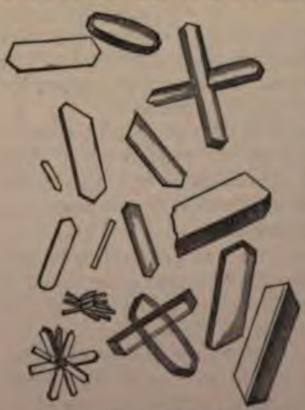

Fig. 18.—ALLANTOÏN.

[1] A drop of fresh urine, mixed with hydrochloric acid, may be observed under the microscope to deposit uric acid crystals in the course of a few minutes.

residue will be obtained, which owes its colour to the formation of alloxantin, $C_8H_6N_4O_8$. On inverting the capsule over another containing ammonia, or otherwise subjecting the residue to ammoniacal vapours, it acquires a magnificent purple colour, owing to the formation of murexide or ammonium purpurate, $NH_4.C_8H_4N_5O_6$. On now adding caustic soda, the purple becomes changed to blue, the colour disappearing on warming. Somewhat analogous reactions are given by caffeine, theobromine, guanine, and xanthine, but the differences do not allow of their confusion with uric acid.

The nitric acid prescribed in the above test may be advantageously replaced by bromine-water, or the material to be tested may be evaporated with a few drops of strong hydrochloric acid and a minute crystal of potassium chlorate.

Uric acid is completely precipitated from its solutions by phospho-tungstic acid in presence of hydrochloric acid.

If uric acid be dissolved in a solution of sodium carbonate, and a drop of the liquid placed on filter paper previously moistened with silver nitrate, a yellow, brown or black spot will be produced, owing to the fact that silver carbonate is reduced by uric acid even at the ordinary temperature.

On adding a little Fehling's solution to a solution of uric acid in caustic soda, a greyish precipitate is formed, which is said to consist of cuprous urate; but with excess of the reagent, and on application of heat, red cuprous oxide separates, and allantoïn is formed.

Uric acid does not reduce a hot alkaline solution of picric acid. This fact distinguishes it from creatinine, glucose, and other normal and occasional constituents of urine which react with Fehling's solution.

For the detection of traces of uric acid in blood or similar liquids, from 150 to 300 c.c. should be boiled, so as to coagulate albumin, and filtered. The filtrate is evaporated at 100° C., and the residue completely exhausted with alcohol. The insoluble portion is then boiled with water, which will dissolve any urates present. The solution is cautiously concentrated, and acetic acid added in excess, when uric acid, if present, will gradually separate, and can be recognised by its microscopic characters and its reaction with nitric acid.

If the quantity of liquid available for the test be very small, 5 or 10 c.c. may be treated in a watch-glass with 6 to 12 drops of strong acetic acid, and a cotton-thread introduced. After standing for twenty-four hours in the cold, microscopic crystals of uric acid may be detected on the thread.

For the *determination* of uric acid in urine, the usual plan is to mix 300 c.c. of the fluid with 15 to 20 c.c. of strong hydrochloric acid, and allow it to stand in the cold for thirty-six to forty-eight hours, when the liquid will be covered with small brownish, reddish, or violet crystals of uric acid, and similar crystals will be found deposited on the sides and bottom of the glass. Examined under the microscope, the crystals mostly present the forms shown in fig. 19B, page 377.

The precipitate should be collected on a smooth filter, washed twice with cold water, then washed with spirit till free from acid, dried at 100°, and weighed on the filter. Or instead of drying the uric acid on the filter, it may be washed off with hot water, the liquid evaporated in porcelain, and the residue weighed.

It is desirable to concentrate the urine by evaporation before employing the foregoing process. According to A. Petit, it is not necessary to allow so long a time for the separation of the uric acid. He strongly agitates 200 c.c. of the urine with 5 c.c. of fuming hydrochloric acid for five minutes, and then allows the liquid to stand one hour. The precipitate is collected on a double weighed filter, dried at 100°, and weighed. Any ash left on ignition should be deducted from the weight of uric acid obtained.

Instead of treating the urine at once with hydrochloric acid, it is often desirable to boil the liquid or substance with milk of lime as long as ammonia is evolved, and treat the filtered liquid with hydrochloric acid as above.

In using the hydrochloric acid method of precipitating uric acid, a correction of 0·0038 gramme should be made for every 100 c.c. of mother-liquor and aqueous washings. Even then the method is liable to give results which are often considerably below the truth, and are responsible for the low figures for uric acid excretion quoted in many text-books. But under the most favourable circumstances, and however carefully the process be conducted, the separation of the uric acid is incomplete, and th results consequently below the truth.

A preferable method of isolating the uric acid from uri is that based on the insolubility of the acid ammonium urat $C_5H_3(NH_4)N_4O_3$, in a solution of ammonium chloride or sulphat In the original process, which is due to A. P. Fokker (abst. *Jou Chem. Soc.*, xxviii. 1293; see also Salkowski, *Fresenius' Ze schrift*, xvi. 371), only a limited amount of ammonium sulpha was used, and hence a considerable correction was necessary f the solubility of the acid urate; but F. Gowland Hopki (*Chem. News*, lxvi. 106) has pointed out that by saturating t liquid with ammonium chloride no such correction is requir and the time necessary for complete precipitation is much reduced. Hopkins prescribes the following procedure :—

To 100 c.c. (or 4 fluid ounces) of urine, finely powdered ammonium chloride is added in excess,[1] about 30 grammes (1 oz.) being necessary. When a small quantity of the salt remains undissolved, even after brisk stirring at intervals of a few minutes, saturation is sufficiently complete, even if complete solution occurs when the liquid recovers from the depression of temperature caused by the solution of the ammonium chloride. The liquid is allowed to stand for two hours with occasional stirring, and is then passed through a thin filter and washed twice with a saturated solution of ammonium chloride. When time is an object, the urine may be made alkaline with ammonia after saturation with ammonium chloride. The phosphates which are thus precipitated with the acid ammonium urate occasion no inconvenience, while precipitation is complete in ten minutes.

When a normal acid urine is saturated with pure ammonium chloride, the precipitate of acid ammonium urate, after being washed with the cold, saturated solution of ammonium chloride, yields a mere trace of ash on ignition, showing that no mineral salts are carried down. Of the ordinary constituents of urine, uric acid, xanthine, and certain pigments appear to be the only bodies precipitated. *Xanthine* is thrown down still more completely from ammoniacal solutions, but it is left in solution when the ammonium urate is subsequently decomposed by hydrochloric acid. *Hypoxanthine* and *creatinine* are not precipitated by ammonium chloride. Certain *pigments* are thrown down, so that the precipitate is always more or less coloured. *Hæmato-porphyrin*, in particular, is very perfectly precipitated, but remains in solution when the urate is subsequently decomposed by acid.

The acid ammonium urate, isolated in the foregoing manner, admits of several alternative treatments, as follow :—

1. When it is desired to determine the uric acid by weight, the precipitate is rinsed off the filter with a jet of hot water, and the liquid heated just to boiling with excess of dilute hydrochloric acid. The liquid is thoroughly cooled and allowed to stand for two hours. It is then filtered on to a smooth filter, and the crystals of uric acid washed twice with cold water, then with alcohol till the washings are no longer acid, dried at 100° C., and weighed.

---

[1] When it is intended subsequently to decompose the precipitate by hydrochloric acid and weigh the liberated uric acid, it is essential that the ammonium chloride used should dissolve to an absolutely clear solution in water, since the quantity employed is very large relatively, and any insoluble matter would seriously vitiate the estimation of uric acid.

To the weight of uric acid thus obtained 0·001 gramme should be added for every 15 c.c. (= $\frac{1}{2}$ oz.) of mother-liquor, the bulk of which need never exceed 30 c.c.; but no correction need be made for the insignificant trace of uric acid dissolved by the aqueous and alcoholic washings. The uric acid thus isolated is usually only slightly coloured and is practically pure. When derived from highly pigmented urines, the uric acid may retain so much colouring matter as to suggest the presence of an amount of impurity sufficient to vitiate the result. In such case, after washing the precipitate of acid ammonium urate off the filter, rectified spirit equal in bulk to the water present should be added, and, after adding hydrochloric acid, the beaker should be covered and heated for some time on the water-bath.

Instead of weighing the uric acid isolated in the foregoing manner, it may, if preferred, be dissolved in a little hot solution of sodium carbonate, and the liquid treated by process 3 or 4.

2. The precipitate of acid ammonium urate having a perfectly definite composition, it may be titrated with standard alkali and an indicator giving no reaction with uric acid. Such an indicator exists in methyl-orange. The precipitate from 200 c.c. of urine is treated with a known measure, *e.g.*, 20 c.c., of decinormal hydrochloric or sulphuric acid, the liquid boiled for some minutes, cooled, diluted to 200 c.c., a few drops of a 1 per cent. aqueous solution of methyl-orange added, and $\frac{1}{20}$-normal caustic soda (=2·0 grammes of NaHO per litre) dropped in from a burette until the orange colour of the acid liquid becomes yellow, which change indicates the point of neutrality. The difference between the volume of acid employed and that of the alkali required to neutralise it represents the ammonia of the precipitate, uric acid having no action on methyl-orange. Each centimetre of $\frac{N}{20}$ solution of soda shows the presence of 0·0084 gramme of uric acid.

3. An alternative plan, when the acid urate of ammonium is not very strongly coloured, is to rinse the precipitate off the filter with hot water, cool the solution, and dilute it with distilled water to 100 c.c. Twenty c.c. of pure concentrated sulphuric acid is then added, so as to acidify the liquid and raise its temperature to about 60° C. (=140° F.), and then a standard solution of potassium permanganate is run in, till the liquid acquires a pink tint surviving agitation and lasting some seconds. Further decolorisation may occur on standing, but this should be disregarded. Each centimetre of $\frac{1}{20}$-normal permanganate (=1·578 gramme of $KMnO_4$ per litre) decolorised represents 0·00375 gramme of uric

acid.[1] F. G. Hopkins strongly recommends this process (*Jour. Path. & Bacteriology*, June 1893). When it is intended to titrate the acid ammonium urate with standard permanganate in the above manner, it is very desirable to wash the precipitate with a saturated solution of ammonium sulphate, instead of ammonium chloride, since the latter salt somewhat affects the accuracy of the titration.

The same method of titration by permanganate may be applied to the uric acid isolated in process 1, after simply dissolving it in a little hot solution of sodium carbonate.

E. Riegler (*Zeit. anal. Chem.*, 1896, xxxv. 31; abst. *Jour. Chem. Soc.*, 1896, ii. 277) boils the acid ammonium urate with Fehling's solution, and estimates the copper in the precipitate obtained.

4. Bayrac (*Compt. rend.*, cx. 352) determines uric acid by evaporating 50 c.c. of the urine to dryness at 100° C., treating the residue with 10 c.c. of dilute hydrochloric acid (1 : 5), and washing with alcohol to remove urea and creatinine. The residue is dissolved on the water-bath in 20 drops of caustic soda solution, and heated to 90° or 100° C. with 15 c.c. of a concentrated solution of sodium hypobromite in the apparatus for estimating urea shown in fig. 10, page 268. 22·38 c.c. of nitrogen measured at 0° C. and 760 mm. barometric pressure, or 23·55 c.c. at the ordinary temperature and pressure, are said to represent 0·084 gramme of uric acid.

Experiments made in the author's laboratory to test the possibility of estimating uric acid by measuring the amount of nitrogen evolved on treatment with alkaline hypobromite have not yielded encouraging results, the reaction being subject to variations from causes not yet understood.

5. Uric acid can be determined by heating it with concentrated sulphuric acid, and determining the ammonia by treatment with hypobromite or distillation with alkali. The method can be applied to a uric acid precipitate.

F. W. Pavy (*Trans. Royal Med. and Chirurg. Soc., London*) has proposed to determine uric acid volumetrically by an ammoniacal cupric solution, in the manner so successfully employed for glucose. Pavy finds the weight of uric acid requisite to decolorise 20 c.c. of the ammoniated Fehling's solution to be 0·01866 gramme, a quantity corresponding to the ratio $C_5H_4N_4O_3 : 3CuO$. Other chemists have found the reducing power of uric acid on Pavy's solution to vary so greatly with the time occupied in conducting the titration and other working conditions as to render the results of very little value.

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[1] This factor is due to F. G. Hopkins, as the result of experiment. As it corresponds to no simple reaction, the process has been investigated in the author's laboratory. The first results on pure uric acid were not very constant ranging from 96 to 104 per cent. of the truth, but on taking as the end-reaction the point at which the permanganate ceased to be *instantly* decolorised, much closer figures were obtained. When the titration was conducted at a boiling heat, instead of at 60° C., the results were higher.

J. B. Haycraft (*Brit. Med. Jour.*, Dec. 1885) has described a process for determining uric acid dependent on its precipitation as silver urate, insoluble in water, ammonia, or acetic acid, on adding a solution of ammonio-nitrate of silver. Haycraft redissolves the precipitate in dilute nitric acid, and determines the silver in the solution by Volhard's thiocyanate method.[1] Czapek prefers to determine the excess of silver present in the filtrate, and Denigès (*Bull. Soc. Chim.*, [3], xi. 226) has described a process on the same principle. Both modifications of the method assume the composition of the precipitate to be constantly Ag_2Ur, which is denied by Salkowski. It is admitted that the silver urate is unstable, metallic silver gradually separating, but it is found by Haycraft and confirmed by Herrmann that if the urine be treated with bicarbonate of sodium and then ammonia before adding the ammoniacal silver solution, this source of error is insignificant, especially if the troublesome filtration of the gelatinous precipitate of silver urate be hastened by the use of an asbestos-filter and a filter-pump. Bodies of the xanthine-group are precipitated by the ammonio-silver nitrate together with uric acid, so that the results obtained are somewhat above the truth; but the method is fairly rapid and simple, and appears to be well suited for comparative clinical observations. The presence of sugar or albumin in the urine does not interfere.

E. Salkowski (*Pflüger's Archiv.*, v. 210) found that the uric acid of urine was not completely precipitated by hydrochloric acid, and that a further amount could be obtained from the filtrate by adding ammonio-silver nitrate. The precipitate consisted of a double urate of silver and magnesium. If the amount of the latter metal present was insufficient to the double salt, unreliable figures were obtained, since silver urate itself either does not exist or is very unstable and of variable composition. Accordingly, in compliance with a suggestion of Salkowski's, E. Ludwig (*Chem. Centralb.*, 1885, page 523) precipitates the urine with a mixture of ammoniacal nitrate of silver and magnesia mixture.

The precipitate, which contains all the phosphoric acid as ammonio-magnesium phosphate, and the uric acid as silver-magnesium urate, is washed with very dilute ammonia and then decomposed by 10 c.c. of a warm dilute solution of potassium sulphydrate. The resultant solution of potassium urate is filtered off, slightly acidulated with hydrochloric acid, and evaporated to a small bulk on the water-bath. The uric acid which separates on cooling is filtered on to glass wool, washed with cold water, dried at 110° C., freed from sulphur by treatment with carbon disulphide, and weighed.

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[1] 1 c.c. of centinormal solution of thiocyanate required by the silver of the precipitate represents 0·00168 gramme of uric acid.

F. G. Hopkins has pointed out (*Chem. News*, lxvi. 107) that treatment with a hot solution of alkaline sulphide is liable to decompose a portion of the uric acid. E. Groves has proposed to decompose the silver urate with potassium iodide instead of with sulphide. Hopkins finds this plan to give too low results, owing to the action of traces of free iodine on the uric acid when the filtrate is acidulated with hydrochloric acid. But any error from this cause could be readily avoided by adding a little sodium sulphite, thus preventing the liberation of free iodine.

As the result of an investigation of the foregoing methods of determining uric acid, W. Camerer (*Zeit. Biol.*, xxvi. 84; *Jour. Chem. Soc.*, lvi. 1040) proposes the following process:—The urine of twenty-four hours, as fresh as possible and preferably free from deposits, is mixed with a known measure of very dilute caustic soda solution and the precipitate of phosphates filtered off. The filtrate is diluted to a gravity of about 1010, or less if rich in uric acid, and 300 c.c. treated with 50 c.c. of a magnesia mixture made from 1 part of crystallised magnesium sulphate, 2 of ammonium chloride, 4 of ammonia of 0·924 sp. gravity, and 8 parts of water. The liquid is filtered, and one-half (=175 c.c.) of the filtrate (avoiding the first portions) is treated with about 0·5 gramme of finely divided calcium carbonate and 5 c.c. of a three per cent. solution of silver nitrate. After ascertaining that the silver solution has been added in excess, the precipitate is filtered off, washed free from silver and chlorides, and dried. It is then treated by Kjeldahl's method for the estimation of nitrogen, and the uric acid calculated from the result obtained.

Arthaud and Butte have described a process of estimating uric acid based upon its precipitation by a standard solution of cuprous thiosulphate. P. Ducong has described a slightly modified form of the same process (*L'union pharm.*, July 1893, page 3299). P. Balke (*Jour. prakt. Chem.*, [2], xlvii. 537) states that the method is feasible when the pure substance is available, but is valueless for the examination of urine.

Urates.

Uric acid is a feeble acid which is usually stated to possess a dibasic function. But it was shown by Bence Jones (*Jour.

Chem. Soc., xv. 201), and has been confirmed more recently by Sir Wm. Roberts (*Croonian Lectures*, 1892), that a third series of urates exist and have great physiological significance. The salts of the formula $M_2C_5H_2N_4O_3$, commonly called *neutral* or *normal urates*, dissolve readily in water, and are exclusively laboratory-products, not being met with in the animal system under either healthy or pathological conditions. The *acid urates*, or "bi-urates," of the formula $MH(C_5H_2N_4O_3)$ are very sparingly soluble, and exist in the urine only after it has undergone ammoniacal fermentation. They are known pathologically as components of gouty concretions in the tissues, but it is questionable if they ever exist physiologically in the blood or tissues. The third class, or "quadri-urates," have the composition $MH(C_5H_2N_4O_3),H_2(C_5H_2N_4O_3)$. They are more soluble than the bi-urates, and are specially the physiological combinations of uric acid. They exist normally in the urine, and probably also in the blood, and constitute the whole of the urinary excretion of birds and serpents. Roberts considers that all the morbid phenomena due to uric acid probably arise from secondary changes in the quadri-urates.

QUADRI-URATES, $MHUr,H_2Ur$, usually present themselves as amorphous powders, but the spheres of birds' and serpents' urine are distinctly crystalline, and display a black cross when examined by polarised light.[1] These forms are permanent in the air if kept perfectly dry, but readily assume a gelatinous character, and then appear under the microscope as large translucent globules. The quadri-urates are difficult to obtain pure. When produced artificially, they are apt to be mixed with free uric acid or bi-urates, and when prepared from urine to be contaminated with pigments and traces of extraneous saline matters. Roberts prepares potassium quadri-urate by adding 2 grammes of uric acid to a boiling solution of 9 grammes of potassium acetate in 300 c.c. of water. The liquid is agitated for about a minute, filtered hot, and cooled rapidly in a stream of cold water. The voluminous precipitate which forms is filtered off, washed in succession with rectified spirit and absolute alcohol, and dried at a temperature not exceeding 40° C. The results of the analysis of the product obtained agree well with the formula $KH(C_5H_2N_4O_3),H_2(C_5H_2N_4O_3)$. Other quadriurates can be obtained by similar means, but they are less stable than the potassium salt.

The quadri-urates are insoluble in alcohol, ether, chloroform, glycerin, and volatile oils, and cannot be dissolved without change in any simple menstruum. When treated with hot water, they pass momentarily into solution, but are almost immediately decomposed into bi-urate and free uric acid. The same decomposition is effected by neutral saline solutions, but in this case, and notably with a solution of common salt, the decomposition is greatly retarded. When treated with solutions of alkaline carbonates, or disodium hydrogen phosphate, the quadri-urates are converted into bi-urates. In healthy urine of feeble acid reaction the quadri-urates dissolve unchanged, but the solution undergoes gradual but complete decomposition, with ultimate separation of the whole of the uric acid in a free state. This change is retarded in normal urine by the salts and colouring matters present (urea has no influence), but occurs with the greater facility the larger the proportion of free acid there is present.

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[1] See Ebstein and Nicolaier, abst. *Jour. Chem. Soc.*, 1891, page 760.

In studying the action of water on quadri-urates, Sir W. Roberts recommends that about 0·4 gramme of the dried deposit should be stirred up with 400 c.c. of distilled water, the mixture heated nearly to the boiling-point until solution is complete, and then left at rest for forty-eight hours. The supernatant liquid is then syphoned off and the remainder passed through a weighed filter. The crystals of uric acid are washed very sparingly with cold water, then more freely with rectified spirit, dried, and weighed.[1] A correction of 0·0055 gramme per 100 c.c. of mother-liquor is applied to compensate for the solubility of the uric acid in cold water. The decanted liquid, filtrate, and washings are mixed together, and the mixed liquid heated nearly to boiling, strongly acidulated with hydrochloric acid, and allowed to stand forty-eight hours as before.

By the foregoing method, Roberts obtained the following figures from two specimens of quadri-urate prepared by the potassium acetate process:—

	Sample A.	Sample B.
Uric acid separated by water (corrected for solubility),	0·080 gramme.	0·164 gramme.
Uric acid dissolved as bi-urate,	0·077 ,,	0·159 ,,

These results sufficiently establish the existence of the quadri-urate and the manner of its decomposition by water.

The decomposition of sodium quadri-urate under the influence of water can be conveniently observed by filtering off the buff-coloured sediment deposited by healthy urine, washing it thoroughly with cold rectified spirit, and drying it at a blood-heat. When the quadri-urate thus purified is mixed with a considerable volume of water it is speedily disintegrated, a portion passing into solution in combination with the bases, and the remainder falling as an insoluble precipitate of crystalline uric acid. The change is readily observed under the microscope by intimately mixing a particle of the purified deposit on a glass slide with a drop of water and protecting the mixture with a covering-glass. In the course of ten minutes ovoid leaflets of uric acid make their appearance, and grow and multiply till in the course of half an hour the entire field is thickly studded with crystals; the process continuing, provided that water be added as required, until the amorphous substance is entirely replaced by crystals of uric acid.[1]

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[1] It is evident that the modified methods of determining uric acid, described on page 363 *et seq.*, may with advantage be employed here.

The quadri-urates readily assume a gelatinous form. Thus, if a 5 per cent. solution of ordinary sodium phosphate be heated to boiling with excess of uric acid, and the liquid filtered hot, the filtrate sets to a jelly on cooling. This jelly, after being pressed between blotting-paper to free it from mother-liquor, exhibits the characteristic behaviour of a quadri-urate, being rapidly decomposed by water with copious formation of crystals of uric acid. On keeping in a moist condition, gelatinous sodium quadri-urate gradually passes into a crystalline condition, and then appears under the microscope in radiating spheres, exactly similar to the spheres so common in serpents' and birds' urine.

If the white mortar-like substance which constitutes the urinary excretion of birds and serpents be examined in its fresh and uncontaminated state, and not after contact with water or bacterial fermentation, it will be found to behave in an exactly similar manner to artificial quadri-urates. Under the microscope it appears as minute spheres which exhibit a radiated structure and display a black cross with polarised light. On adding a drop of water, the spheres are seen gradually to melt away, with formation of hexagonal tablets of uric acid.

According to W. Roberts, the urinary secretion of serpents and birds consists almost wholly of quadri-urates, and he gives the following analysis of that from the boa, taken from the interior of a massive and very pure specimen[2]:—

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[1] Crystals of the sodium bi-urate simultaneously formed are never observed, since this salt is liberated in the gelatinous form.

[2] Roberts points out that the boa and other large serpents void their urine at long intervals, varying from a week to six or seven weeks, and that during its long sojourn in the urinary passages the secretion undergoes changes in its composition, with liberation of free uric acid. On this account, the uric acid separated by treatment with water is always notably in excess of that which goes into solution as bi-urate. This is shown by the following figures obtained by the treatment of freshly gathered specimens of boa's urinary excrement, with water:—

	A	B	C	D
Uric acid separated by water, . .	0·128	0·110	0·204	0·215
Uric acid dissolved as bi-urate, .	0·117	0·085	0·141	0·140

In addition, contact with the water used for cleansing the cages, and bac-

Uric acid,	82·80	per cent.
Potassium,	3·33	"
Sodium,	1·06	"
Ammonium,	1·92	"
Moisture, organic matter, iron, traces of lime, and loss,	10·89	"
	100·00	"

Uric acid as quadri-urates,	80·71	per cent.
Uric acid in the free state,	2·09	"

BI-URATES have the general formula, $MH(C_5H_2N_4O_3)$, or MHUr. They result from the action of water on the quadri-urates, and exist in the body under various pathological conditions. The sodium salt, which is the most important and characteristic member of the series, possesses the following properties:—

Acid Sodium Urate, or *Sodium Bi-urate*, contains $2(NaHC_5H_2N_4O_3)+H_2O$. It generally forms a crystalline powder, which, under the microscope, appears in needles (often crossed), rosettes, stellate and hedgehog-like forms (fig. 20, page 378). It requires about 1200 parts of cold or 120 of boiling water for solution. Sodium bi-urate is readily obtained by passing carbon dioxide through a solution of uric acid in caustic soda, or by boiling uric acid with sodium carbonate, phosphate, or acetate, or with borax. The buff or brick-red sediment often thrown down by urine is commonly stated to consist of sodium bi-urate, but Roberts has shown that it consists essentially of sodium quadri-urate (page 369).

The solubility of acid urate of sodium in water impregnated with salt and other substances has an important bearing on the cause and cure of gout, and has been investigated by Roberts, from whose results it appears that the solvent action of the various salts depends on the nature of the metal, and has no reference to its form of combination. Salts having an alkaline reaction to litmus, like the carbonates and phosphates, behave exactly similarly to those of neutral reaction, such as the chlorides and sulphates. Salts of potassium exert no appreciable influence on the solubility of sodium bi-urate in water. Salts of sodium decrease the solubility, the influence being greater the larger the proportion of salt present. Salts of ammonium, calcium, and magnesium behave similarly to, but less powerfully than, salts of sodium.

terial decomposition owing to imperfect desiccation, cause further change of boa's excrement as met with in commerce, so that the article usually met with consists essentially of bi-urate of ammonium.

The following figures by Sir Wm. Roberts represent the parts by weight of sodium urate dissolved at 100° F. (=37·8° C.) by 1000 parts of the solutions of the strengths indicated. The amount of sodium bi-urate dissolved by 1000 parts of distilled water at 100° F. was found to be 1·0.

Percentage of Salt in Solvent.	0·1 per cent.	0·2 per cent.	0·3 per cent.	0·5 per cent.	0·7 per cent.	1·0 per cent.
Sodium bicarbonate,	0·50	0·34	0·20	0·13	0·09	0·08
Sodium chloride,	0·45	0·30	0·16	0·10	0·08	0·05
Sodium phosphate (crystallised),	0·70	..	..	0·32	..	..
Sodium sulphate (crystallised),	0·55	..	..	0·24	..	..
Sodium salicylate,	0·65	..	0·36	0·25	..	..
Potassium bicarbonate,	0·96	1·00	1·00	0·97	1·02	0·98
Potassium chloride,	0·96	..	1·01	1·10	..	..
Potassium phosphate,	1·01	..	..	1·00	..	..
Ammonium chloride,	0·85	0·50	0·42	0·35	..	..
Calcium chloride,	..	..	..	0·27	..	..
Calcium sulphate,	0·65	0·44	..	..	..	..
Magnesium chloride,	0·85	..	..	0·63	..	..
Magnesium sulphate (crystallised),	0·90	..	..	..	..	..

Crystalline sodium bi-urate is ten times as soluble in boiling water as in cold, but a saturated hot solution does not deposit the excess of salt immediately on cooling. The bi-urate remains in complete solution for a considerable time, and is not entirely deposited for some days. Roberts has shown that this behaviour is not merely due to supersaturation of the liquid, but is owing to the formation of a gelatinous modification of the bi-urate of greater solubility than the crystalline form. Thus if a saturated solution of sodium bi-urate in boiling water be prepared, and when cold mixed with an equal measure of a 20 per cent. solution of common salt, a voluminous gelatinous precipitate will be thrown down. Saturated solutions or solid crystals of other salts (*e.g.*, sodium phosphate or acetate, potassium chloride, phosphate, acetate, &c.) may be substituted for the common salt. The precipitate, if filtered off, allowed to drain, and cautiously washed with cold water, consists of sodium bi-urate in a state of approximate purity. It dissolves at 100° F. in blood-serum, or in a liquid containing 0·5 gramme of sodium chloride and 0·2 of sodium carbonate per 100 c.c. (which represents the saline ingredients of serum), sufficiently freely to cause a considerable separation of uric acid after acidulating with acetic acid; whereas crystalline sodium bi-urate is taken up by water at 100° F. so slightly that no deposition of uric acid is obtainable on acidulating the liquid.

The gelatinous form of sodium bi-urate gradually changes into the crystalline variety, and the gradual deposition of the salt from its solution in water, blood-serum, or imitation-serum is evidently due to the same change of condition.

Acid Potassium Urate, or *Potassium Bi-urate*, KHUr, is said to be sometimes formed as a urinary deposit in cases of fever. It is amorphous, and is more soluble than the corresponding sodium salt, requiring for solution only 800 parts of cold or from 70 to 80 parts of boiling water.

Acid Lithium Urate, or *Lithium Bi-urate*, LiHUr, forms crystalline grains, soluble in 370 parts of cold or 39 of boiling water. Lipowitz states that if equal parts of uric acid and lithium carbonate be treated with 90 parts of water at blood-heat, a clear solution is obtained, while at 100° C. four times the amount of uric acid can be dissolved without increasing the weight of lithium carbonate. Seeing that lithium carbonate itself requires about 200 parts of water for solution, its solvent action on uric acid is remarkable, and is of much interest in connection with the extensive application of lithium salts in the treatment of gout. On the other hand, it is stated by L. Siebold (*Year-book Pharm.*, 1889, page 413), as the result of direct experiment, that the relative solvent action of solutions of lithium, sodium, and potassium carbonates on a given weight of uric acid, under equal conditions of dilution and at a temperature of 37° C. (blood-heat), is strictly proportional to the ratio of the molecular weight of these solvents. Hence lithium carbonate has the advantage that 74 parts are chemically equivalent to 106 of the sodium salt or 138 of potassium carbonate; but there the advantage ceases. Urinary sediments are similarly dissolved by these carbonates with equal facility if molecular proportions are used, and equivalent weights of the citrates of lithium, sodium, and potassium produce equal alkalinity in the urine of the person taking them. Siebold further states that lithium chloride and sulphate have no solvent action on uric acid and acid urates, and that natural mineral waters containing these salts have none beyond that exercised by basic constituents simultaneously present, and by the water (compare pages 198, 253).

Acid Ammonium Urate, $(NH_4)HUr$, is soluble in about 1500 parts of cold water, and quite insoluble in saturated solutions of ammonium chloride and sulphate (compare page 362). The urinary excrement of serpents is commonly stated to consist almost wholly of a mixture of acid urate of ammonium with free uric acid. This is often true of the altered product, but Sir W. Roberts has shown that, in a fresh and undecomposed state, serpents' urine consists substantially of quadri-urates, which undergo decomposition into a mixture of bi-urates and free uric acid by contact with water (page 370). Guano, the excrement of various aquatic birds, consists chiefly of oxalate and acid urate of ammonium in admixture with phosphates. Guanine (page 315) is also a constituent of guano, and replaces uric acid in the urine of spiders and other invertebrate animals.

Piperazine urate is described on page 198.

NEUTRAL OR NORMAL URATES of the light metals do not exist naturally, but they may be obtained by dissolving uric acid in the theoretical amount of alkali. The normal urates of lithium and ammonium are unknown. *Neutral potassium urate*, $K_2C_5H_2N_4O_3$, forms small crystals having an alkaline reaction and caustic taste. It dissolves, with partial decomposition into the acid salt, in about 36 parts of cold water, forming a liquid of soapy taste which froths strongly when shaken. *Normal sodium urate*, $Na_2Ur + H_2O$, forms hard nodules which closely resemble the potassium salt, but are less soluble in water.

On passing carbon dioxide through a solution of the normal urate of potassium or sodium the corresponding acid urate is precipitated. The same decomposition occurs by prolonged boiling of the solution, or by its exposure to air.

The following table, due to Ralfe (*Practical Treatise on Diseases of the Kidneys*, 1885), shows the character of the urates of the light metals :—

Urate.	Solubility in Cold Water.	Character of Deposit.
Potassium; acid, . .	1-800	Amorphous.
" normal, . .	1-44	Amorphous, or in fine needles.
Sodium; acid, . . .	1-1200	Amorphous; rarely crystalline.
" normal, . .	1-77	Nodular masses.
Lithium; acid, . . .	1-60	Amorphous, or in fine needles.
Calcium; acid, . . .	1-600	Amorphous, or in fine needles.
" normal, . .	1-1500	Fine granules.
Ammonium; acid, . .	1-1600	Amorphous, or spiked globular masses.

Calcium urate is a frequent constituent of gouty deposits (S. Delápin, *Jour. Chem. Soc.*, lii. 469).

The urates of lead, copper, mercury, and silver are quite insoluble in water. Hence solutions of these metals are used for determining uric acid or for separating urates from urine (compare page 366).

The behaviour of the urates with water and saline solutions has an important bearing on the cause and treatment of gout. It is probable that in media containing alkaline carbonates—such as the serum of the blood, and its derivatives lymph and synovia—uric acid passes into solution in the first instance as quadri-urate, and it may be inferred that it circulates in the blood and is voided in the urine in the same form. In perfect health, the elimination of the quadri-urate proceeds with sufficient speed and completeness to prevent any undue detention or any accumulation of it in the blood. But in gouty subjects, either from defective action of the kidneys or from excessive introduction of uric acid into the circulation, the quadri-urate lingers unduly in the blood and accumulates therein. The detained quadri-urate, circulating in a medium rich in carbonate of sodium, gradually takes up an additional atom of base, and is thereby converted into bi-urate, which at first exists in the hydrated or gelatinous condition, but with lapse of time and accumulation passes into the insoluble crystalline condition, and then the symptoms of gout manifest themselves.[1]

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[1] A. Haig (*Med. Chirurg. Trans.*, lxxi. 125, 283) has shown that administration of acids diminishes the relative amount of uric acid excreted, while that of alkalies increases it. Thus the normal proportion of uric acid to urea is 1:35, but after a few doses of citric acid the relation was 1:41, and after similar doses of potassium citrate 1:28. In these cases there was not only a relative but also an absolute diminution or increase in the uric acid excreted. Salicylic acid forms an important exception to the general behaviour of acids, for while it increases urinary activity, it does not in any way diminish the excretion of uric acid. Moreover, acids given while salicylates are present in the circulation have no longer the power of diminishing the excretion of uric acid, nor is excessive excretion of uric acid under salicylates accompanied by any headache. Both uric and salicyluric acids are present in the urine passed under the influence of salicylates, probably owing to the salicylate acting on the uric acid in the blood, but not on that secreted by the kidney itself. Benzoates do not act in the same way as salicylates, probably because the hippuric acid formed from them is less soluble than salicyluric acid. The value of salicylates in uric acid diseases is largely due to their power of preventing acids from causing retention of uric acid. Thus, according to Haig, salicylates prevent gout, the peculiar headache due to uric acid and frequent after breakfast, and also epilepsy, which last affection he believes to be due to uric acid acting on the nerve-centres.

Urinary Deposits and Calculi.

Urine is sometimes turbid as passed from the urethra, and all urine deposits a fine cloud of mucus on standing. Many specimens of urine, when allowed to cool and stand, deposit urinary salts. These sediments may appear as a purely amorphous or crystalline precipitate, or as a mixture of amorphous and crystalline particles.

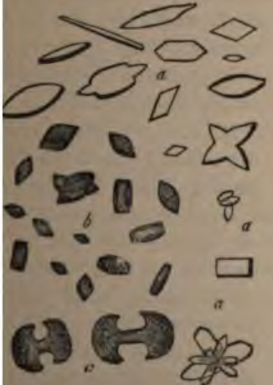
Urine which is turbid when actually passed from the urethra (and consequently at the temperature of the body) may owe its turbidity to the presence of suspended *uric acid* or *urates*; to the presence of *earthy phosphates* or *carbonates* (especially if the urine be that of a herbivorous animal); or to the presence of *organised matters*, such as *mucus* or *pus*. Deposits which require removal by surgical means from the kidneys, ureters, bladder or urethra, are best considered separately under the head of Urinary Calculi (page 380).

Uric acid and urates are by far the most common and abundant constituents of urinary sediments and calculi.

URINARY SEDIMENTS.

Urinary deposits are rarely of a complex character, and hence very simple methods suffice to determine their nature. Examination under the microscope is specially suited for this purpose, since it is simple and readily applied, and is available for a very minute quantity of sediment. The urine to be examined should be allowed to stand for twelve hours in a conical glass, so that any deposit may collect at the apex. A drop of the liquid, containing as much sediment as possible, should then be withdrawn by a pipette, and placed on a microscope-slide. It should be covered with a thin glass, and examined under an inch power, which may subsequently be changed for a $\frac{4}{10}$ or $\frac{1}{4}$-inch power. Epithelium, mucus-globules, and pus-cells will be distinguished as organised deposits. Urates and amorphous phosphates appear as opaque particles. Uric acid, which to the naked eye appears as a coloured sandy deposit, is distinguished under the microscope by its peculiar crystalline form and yellow or brown colour. Earthy phosphates, when crystalline, are well characterised by their forms and by their freedom from colour. Urine which has been allowed to stand for some time frequently contains a deposit of calcium oxalate, which is seen under the microscope as delicate octahedra. On adding a drop of normal caustic alkali on the slide, a deposit of uric acid will at once dissolve. Normal hydrochloric acid, on the contrary, leaves uric acid unaffected, or causes a further deposition of minute leaflet crystals under the eye of the observer, while it at once dissolves earthy phosphates or carbonates, whether stalline or amorphous. The organised deposits are more or less iefied by alkali, but dilute acid leaves them unchanged. The lition of a minute amount of staining material, either finely vdered or in solution, at the bottom of the depositing vessel, l stain epithelium and other organised deposits, and thus ilitate their recognition under the microscope.

Uric acid appears under the microscope in a variety of forms. adratic prisms, single and in groups, spiculæ, aigrettes, and imb-bell" forms are common, ilso are somewhat oval crys- attached together so as to m figures of eight, stars, or sses (fig. 19, A). From urine lulated with 5 per cent. by isure of hydrochloric acid, are crystals are deposited, ing two opposite sides smooth the alternate sides jagged . 19, B). Uric acid crystals solve on adding caustic alkali, are reprecipitated in minute characteristic forms on sub- iently adding hydrochloric l.



g. 19, A.—Crystals of Uric Acid. —*a*, From decomposition of urates; *b*, from human urine; *c*, dumb-bell forms.

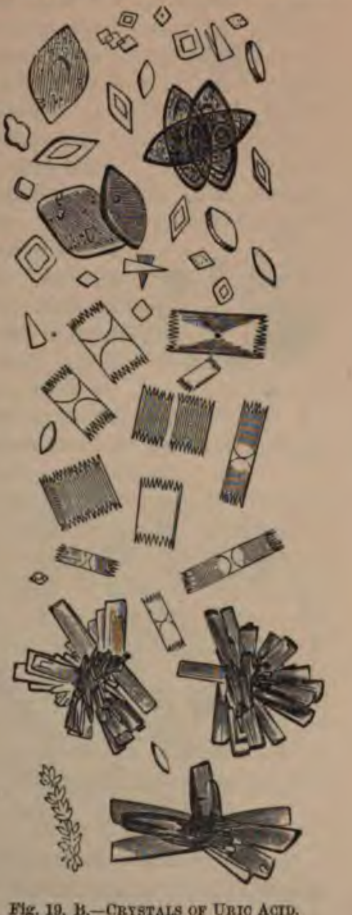


Fig. 19, B.—Crystals of Uric Acid.

cid sodium urate usually forms amorphous deposits, but some- es occurs as bundles or tufts of acicular crystals, or in spheroidal masses (fig. 20). *Potassium* and *magnesium urates* are almost always amorphous. *Ammonium urate* occurs only in alkaline urine, and generally in association with ammonio-magnesium phosphate. It forms irregular, club-like crystals or thorn-apple spherules (fig. 21). Urates are readily distinguished from phosphates by their solubility when warmed in their supernatant urine. When treated with acetic acid, deposits of phosphates dissolve, but urates change into characteristic forms of uric acid without previously undergoing solution.

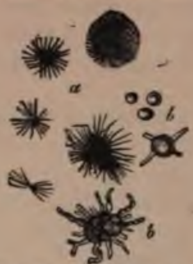


Fig. 20.—Acid urate of sodium.—*a*, needles, usually aggregated; *b*, *b*, spheroidal masses.

Hippuric acid, according to Gorup-Bezanez, is occasionally met with in sediments from the urine of human patients who have taken benzoic acid. It occurs frequently in sediments from the urine of herbivorous animals. Hippuric acid forms characteristic acicular crystals and rhombic prisms (see fig. 24, page 388). Some of the broader crystals resemble those of ammonium magnesium phosphate, but are insoluble in acetic acid. From uric acid they are distinguished by their solubility in alcohol. If the alcoholic solution be evaporated to dryness, and the residue dissolved in warm water, characteristic crystals of hippuric acid will be obtained on evaporation.

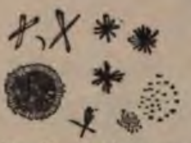


Fig. 21.—Acid ammonium urate.

Ammonium magnesium phosphate, often described as "triple phosphate,"[1] is deposited from urine as soon as the liquid becomes alkaline from the decomposition of the urea. The natural deposit may be imitated by adding ammonium carbonate to urine, filtering the liquid immediately, and allowing the filtrate to stand for some hours. Ammonium magnesium phosphate forms fine, vitreous, prismatic crystals ("coffin-shaped"), or ragged arborescent or stellate forms (fig. 22).

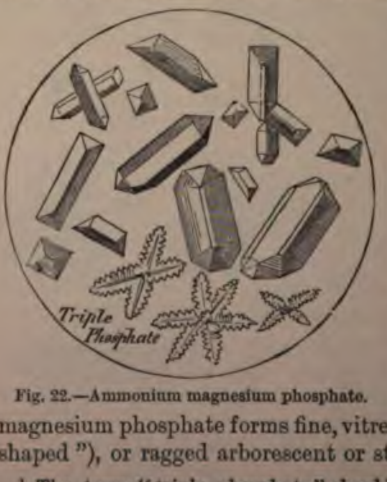


Fig. 22.—Ammonium magnesium phosphate.

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[1] The term "triple phosphate" should be abandoned as unscientific and misleading.

Calcium phosphate occurs commonly as an amorphous deposit, which to the naked eye resembles pus or granular organic matter. When precipitated from the urine by heat this deposit has been mistaken for albumin, but is distinguished therefrom by readily dissolving on adding a drop of acetic acid. The same character, and its insolubility in the supernatant urine on warming, distinguish it from deposits of amorphous urates. Under the microscope, amorphous calcium phosphate appears as minute pale granules, arranged in irregular patches. *Magnesium phosphate* has a similar microscopical appearance and characters. The crystalline form of calcium phosphate is a comparatively rare deposit. Under the microscope, it appears as crystalline rods, frequently grouped in stars or rosettes, or in club or wedge-like forms, which always show the lines of crystallisation.

Calcium oxalate usually occurs in minute octahedral crystals or dumb-bell forms (fig. 23), requiring a $\frac{1}{4}$-inch objective for their recognition; it is liable to escape detection unless the phosphates are first dissolved by acetic acid. It occurs in certain morbid conditions, and in the urine of persons who have eaten rhubarb. The deposit may be readily induced by adding a crystal of oxalic acid to normal urine, and allowing the liquid to stand for some hours.

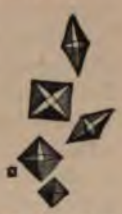

Fig. 23.—Crystals of calcium oxalate.

Calcium carbonate is rarely found as a deposit in human urine, but frequently in that of the horse and other herbivora. Under the microscope, the deposit appears as minute spherules or dumb-bells, which show a well defined black cross when viewed by polarised light. It dissolves in warm acetic acid, with effervescence, which may be observed under a low microscopic power, if a number of crystals be treated beneath a large cover glass.

Calcium sulphate in the form of acicular crystals is said to have been observed as a urinary deposit.

Cystin occurs very rarely as a urinary deposit. It takes the form of hexagonal plates (fig. 5, page 228), which are soluble in ammonia, and are redeposited in a more perfect form by allowing the resultant solution to evaporate spontaneously.

Leucine (page 213), *tyrosine* (page 217), *xanthine* (page 312), and *cholesterin* occasionally occur as urinary deposits.

Organised deposits of various kinds are apt to occur in urine. Blood-corpuscles, epithelium cells, tube casts, pus-corpuscles, fat-globules, and spermatozoa are all more or less common under certain conditions. Their recognition depends on the employment of a microscopic power of 300 or 400 diameters, sometimes with the aid of staining agents.

For the more formal chemical examination of urinary deposits, the turbid urine should be warmed to about 50° C., and filtered at that temperature. Deposits of earthy phosphates, calcium oxalate, uric acid, or organised matters are not dissolved on heating, and hence will be found on the filter, but urates mostly dissolve and are re-deposited from the filtrate in a comparatively pure form after cooling. The uric acid in such deposits may be identified by the murexide test (page 359), while the resultant residue may be ignited and employed for identifying the base. Deposits of urates are often pink or red, owing to the presence of pigment, which may be removed from the deposit by treatment with alcohol.

The portion of the urinary deposit which does not dissolve on warming may be treated with decinormal hydrochloric acid, which will dissolve the earthy phosphates without affecting uric acid or the organised deposits. Or the insoluble portion of the deposit may be warmed with dilute acetic acid, and the liquid filtered. From the acetic acid solution, calcium may be thrown down by ammonium oxalate. If the filtrate be rendered strongly alkaline with ammonia, a white crystalline precipitate, giving streaks in the track of a glass rod, consists of ammonium-magnesium phosphate. If, after standing for some time, the ammoniacal liquid be filtered and treated with a drop or two of magnesia-mixture, and again stirred, a further precipitation of ammonium-magnesium phosphate proves that calcium phosphate was present in the original deposit. The portion of the deposit insoluble in acetic acid may contain uric acid and calcium oxalate. The former is readily detected by the murexide test, by its insolubility in cold hydrochloric acid, and by its microscopic appearance. The calcium oxalate is insoluble in acetic acid, but dissolves in hydrochloric acid, and is reprecipitated on adding excess of ammonium acetate to the resultant solution.

URINARY CALCULI.

Concretions, which in the majority of cases consist essentially of uric acid or urates, frequently occur in the bladder, kidneys, and other parts of the urinary passages. These concretions, or urinary calculi, vary greatly in form, consistency, and composition, and according to their size are known as stones, gravel, or sand. Though sometimes homogeneous, they are more frequently composed of concentric layers, and are always formed on a nucleus, which may generally be distinguished from the adjacent portions and sometimes consists of a foreign body.

Uric acid; ammonium, potassium, sodium, calcium and magnesium urates; iron, calcium, and ammonio-magnesium phosphates; calcium and magnesium carbonates (especially in calculi from herbivora); calcium oxalate; ammonium hippurate; cystin; xanthine; ferric oxide; silica; silicates (in sheep's urine); mucus; blood; colouring and other extractive matters, have all been mentioned as constituents of urinary calculi. It is very unusual to meet with calculi formed exclusively of a single substance. Many calculi are formed chiefly of uric acid, and others chiefly of calcium oxalate; but these concretions generally contain at least a small quantity of other matters. The most common kinds of human urinary calculus are:—*a*, Uric acid, with urates of calcium and ammonium; *b*, ammonium magnesium phosphate, with calcium phosphate and carbonate; *c*, uric acid, with phosphates; and *d*, calcium oxalate, with phosphates. Calculi consisting essentially of cystin, xanthine, and other compounds, are occasionally, but very rarely, met with.[1]

The following analyses illustrate the percentage composition of some typical kinds of urinary calculus:—

URIC ACID CALCULI.

	A.	B.
Uric Acid,	92·8	84·69
Urates,	3·2	9·03
Ammonium magnesium Phosphate,	...	1·12
Extractive Matters,	1·0	2·61
Water,	3·0	

PHOSPHATIC CALCULUS.

Sodium Urate,	9·77
Calcium Phosphate,	34·74
Ammonium magnesium Phosphate,	38·35
Calcium Carbonate,	3·14
Magnesium Carbonate,	2·55
Extractive Matters,	6·87

OXALATE CALCULUS.

Calcium Oxalate,	63·5
Calcium Phosphate,	6·2
Water and Organic Matters,	30·3

J. Horbaczewski (*Zeits. Physiol. Chem.*, xviii. 335) gives the following analyses showing the centesimal composition of certain rare urinary calculi :—

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[1] Recording his experience in 1882, Sir Henry Thompson stated that calculi consisting chiefly of uric acid and urates formed about 60 per cent. of the whole, a few of these having a slight admixture of phosphates; calculi composed of phosphates alone, or together with some uric acid, accounted for about 36 per cent.; and about 3 per cent. of the total number were composed of calcium oxalate. One instance only of cystin calculus and one of calcium phosphate had come within his experience.

	FATTY CONCRETION.	CHOLESTEROL CONCRETION.
Water,	2·5	3·76
Ash,	0·8	0·55
Organic Matters insoluble in ether,	11·7	0·15
Organic Matters soluble in ether,	85·0	95·84
Containing :—		
Free Fatty Acids,	51·5	...
Neutral Fats,	33·5	...
Cholesterol,	traces	95·87

The concentric layers of urinary calculi are frequently distinct in composition as well as in appearance, and a curious alternation of material is at times observed ; uric acid, for instance, changing place with urates, phosphates, oxalates, &c. A nucleus of uric acid is generally enclosed with an external coat of phosphates,[1] but the reverse of this appears never to occur. The exterior layers in calculi of various composition are generally phosphatic. The oxalate calculi are usually the hardest, the phosphatic the softest.

Uric acid calculi are very frequently met with. When composed almost wholly of uric acid, a minute portion, heated on platinum foil, chars, burns, and leaves scarcely a trace of ash. Such calculi are usually brownish-red, smooth, or tuberculated, and are composed of concentric laminæ.

Ammonium urate calculus is uncommon. It is clay-coloured, smooth, and composed of fine concentric laminæ. This calculus is wholly volatile on ignition.

Cystin calculi are very rare. They are usually small, semi-transparent, smooth, of a greenish or brownish-yellow colour, and insoluble in water, alcohol, or ether. They are soluble in ammonia, and the ammoniacal solution leaves the cystin in hexagonal plates when treated with acetic acid or allowed to evaporate spontaneously.

Xanthine calculus is of very rare occurrence. It is pale brown, of a polished appearance, and soluble in alkaline liquids. On treatment with hydrochloric acid a xanthine calculus yields a solution which on cooling deposits xanthine hydrochloride in hexagonal scales (page 313).

Cholesterin often occurs largely in gall-stones or biliary calculi,

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[1] The external layers of phosphate represent a damaged condition of the urinary apparatus consequent upon the growth and presence of the uric acid or other nucleus.

ly forms an essential part of urinary calculi. The
is true of *bile-pigments* and *bile-acids*.
xalate often occurs alone, forming a deep red-brown
y hard calculus, tuberculated on the exterior, and
its appearance "mulberry calculus." Smaller and
etions of calcium oxalate often appear as "hemp-
Calcium oxalate occurs in large quantities in horse's
en as concretions in pig's urine.
hosphate occasionally forms concretions of a pale
, composed of regular laminæ.
ulphate calculus has been met with in only one
.
n magnesium phosphate (improperly called "triple
forms white, brittle, and crystalline calculi having an
ce. It is seldom laminated, and is not very common.
calculus" is a mixture of calcium phosphate and
agnesium phosphate. Such calculi are of frequent
nd derive their name from the readiness with which
ggregates and even fuses to a bead when heated on
e before the blowpipe. The fusibility increases with
n of ammonium magnesium phosphate contained in
calcium phosphate being very infusible. Fusible
rely laminated. They are usually white, soft as chalk,
very large.
Examination of Calculi.
ulus be entire, and sufficiently large to allow of the
ould be sawn in half to ascertain whether it is homo-
uilt up of differing concentric layers. If the latter,
ch layer should be flaked off and examined separately.
ary examination should be made by carefully apply-
ving tests :—
small fragment of the sample on platinum foil, and
esult. Cholesterin melts and burns freely. Fibrin
odour of burnt feathers, and cystin a smell of burning
the calculus consist wholly of uric acid, ammonium
xanthine, cholesterin, or other organic matter, it will
olatilised on ignition. Any residue may consist of :—
xide, or of potassium or sodium carbonate derived from
usly existing; calcium carbonate originally existing
derived from calcium urate or oxalate; calcium or
phosphate; and traces of silica, oxide of iron, &c.
left after ignition should be taken up on a loop of
e moistened with hydrochloric acid, and examined in
ne for the detection of sodium, potassium, and calcium.

2. Treat a second portion of the calculus with a cold solution of caustic alkali. The evolution of *ammonia* points to the presence of ammonium urate or ammonium magnesium phosphate in the calculus. On adding a few drops of lead acetate to the alkaline liquid and boiling, a black precipitate of lead sulphide will be formed if any *cystin* were originally present.

3. Treat a third small quantity of the calculus with warm dilute nitric acid. Any effervescence may be due to decomposed urate or to uric acid, but more probably to the presence of *calcium carbonate* in the calculus. The acid liquid should then be evaporated to dryness on the water-bath. A deep yellow residue points to the presence of *xanthine*, but its presence should be confirmed by the additional tests described on page 314. *Uric acid* and *urates* leave a bright red residue, which on exposure to ammoniacal vapours assumes a magnificent purple tint (page 360).

Careful application of the foregoing tests will generally give adequate information as to the general nature of the calculus, and will suffice to establish the presence or absence of most of the possible constituents. In many cases it is unnecessary to make an exhaustive analysis, but where this is required the systematic process on next page may be advantageously employed. It presupposes the calculus to be of the most complex nature, but the results of the preliminary examination will generally allow the procedure to be materially abridged.

Xanthine and *cystin* are occasional, but rare, constituents of urinary calculi. The latter may be detected by boiling a portion of the calculus with caustic alkali and lead acetate, and the former by its reaction with nitric acid (page 314). When present, xanthine and cystin are precipitated with uric acid when the solution obtained by boiling the calculus with caustic soda is treated with hydrochloric acid. They may be separated from uric acid by treating the precipitate with warm dilute hydrochloric acid. The filtrate, when concentrated and cooled, will deposit the xanthine hydrochloride in crystalline plates. Cystin may be precipitated from the solution as the benzoyl-compound (page 229). Or the calculus, preferably previously exhausted in succession with ether, alcohol, and water, may be treated with warm ammonia. On evaporating the ammoniacal solution nearly to dryness the cystin is deposited in crystalline tables, or it may be precipitated by somewhat concentrating the ammoniacal solution and adding excess of acetic acid. Xanthine may be isolated and precipitated in the same manner. Its co-occurrence with cystin has not been observed, so that no separation of the two bodies is necessary.

<table>
<tr><td rowspan="7">EXTRACT is evaporated. The residue, dried at 100° and weighed, consists of *cholesterin*, *fatty matters*, and certain *resinous biliary matters*.
Boil with alcohol, filter hot, and cool filtrate. Cholesterin will be deposited in crystalline plates having the characteristic angles (79° 30′ and 100° 30′).
To separate cholesterin from fatty matters, &c., saponify with alcoholic potash, boil off the spirit, add water, and shake with ether, which dissolves the *cholesterin* only. The alkaline liquid acidified with dilute sulphuric acid gives *fatty and resinous acids*, which can be shaken out with ether and recovered by evaporation.</td><td rowspan="7">ALCOHOLIC EXTRACT may contain hippuric acid, bile-pigments, and other colouring matters, the weight of which can be ascertained by weighing the residue left on evaporation.</td><td colspan="11">RESIDUE. Remove from the Soxhlet-tube, boil with a moderate quantity of water, and filter hot. If the filtrate gives a deposit on cooling, the residue should be boiled again with water, but not otherwise.</td></tr>
<tr><td colspan="3" rowspan="2">AQUEOUS EXTRACT. Evaporate to small bulk, add excess of hydrochloric acid, and allow to stand in the cold for six hours. Filter, and wash deposit with a little cold water.</td><td colspan="8">RESIDUE. Treat with warm dilute caustic soda, and filter.</td></tr>
<tr><td colspan="2">FILTRATE. Add excess of acetic acid, keep cold for some hours, and filter.</td><td colspan="6">RESIDUE. Wash with a little cold water and treat with warm acetic acid. Effervescence indicates *calcium carbonate*. Dilute the liquid and filter.</td></tr>
<tr><td colspan="2">FILTRATE. Divide into two equal portions.</td><td rowspan="4">PRECIPITATE, washed with alcohol to remove colouring matter, consists of *uric acid* existing in the calculus as *urates*. It may be dried at 100° and weighed, or the uric acid may be determined, without previous precipitation by hydrochloric acid, by the methods on page 363, *et seq.*</td><td rowspan="4">FILTRATE. Divide into two equal portions.
To one portion add potassium ferrocyanide. If a white flocculent precipitate be formed, due to *proteids*, evaporate remaining portion of solution to dryness, heat residue at 100° till free from acetic acid, and add water. The insoluble matter consists of *mucus* and *albuminous matters*.</td><td rowspan="4">PRECIPITATE consists of *uric acid*, existing as such in the calculus, and in rare cases of *xanthine* and *cystin* (see page 384).</td><td colspan="4">SOLUTION. Add ammonium oxalate, digest, and filter.</td><td colspan="2">RESIDUE. Boil with a strong solution of sodium carbonate, dilute, and filter.</td></tr>
<tr><td rowspan="3">I. Distil with lime, and titrate distillate with standard acid. The *ammonia* found existed in the calculus as *acid ammonium urate*.</td><td rowspan="3">II. Evaporate to dryness. The residue consists of NaCl (with perhaps KCl and $MgCl_2$, &c.), corresponding to the *urates* of the calculus.</td><td colspan="3">FILTRATE. Add excess of ammonia, and filter.</td><td rowspan="3">PRECIPITATE of CaC_2O_4 represents the Ca existing in the calculus as *carbonate* and *phosphate*.</td><td rowspan="3">SOLUTION. Acidulate with acetic acid, add $CaCl_2$, digest, and filter. Precipitate, washed, dried at 130°, and weighed, represents the *calcium oxalate* of the original calculus. On strong ignition it gives the CaO existing as oxalate.</td><td rowspan="3">RESIDUE consists of $CaCO_3$ from decomposition of calcium oxalate, together with oxide of iron, silica, &c.</td></tr>
<tr><td colspan="2">FILTRATE. Divide into two equal parts.</td><td rowspan="2">PRECIPITATE washed with ammonia water represents the *ammonio-magnesium phosphate* of the calculus.</td></tr>
<tr><td>I. Add magnesia mixture. Ppte. represents phosphate existing as *calcium phosphate*.</td><td>II. Add sodium phosphate to ppte. remaining *magnesia*, if any.</td></tr>
</table>

Hippuric Acid. Benzoyl-amidoacetic Acid. Benzoyl-glycocine.

$$C_9H_9NO_3;\ \textit{i.e.},\ C_6H_5.CO.NH.CH_2.COOH.$$

Hippuric acid affords a typical example of the so-called "conjugated bodies," the synthesis of which is readily effected within the living organism. Thus, if benzoic acid be taken internally, it appears in the urine as hippuric acid, and hippuric acid may be obtained artificially by heating benzoic anhydride with amidoacetic acid (glycocine), or the zinc salt of the latter with benzoyl chloride :—

$$C_7H_5O.Cl + C_2H_2(NH_2)O.OH = C_7H_5O.O.C_2H_2(NH_2)O + HCl.$$

Benzoyl Chloride. Glycocine. Hippuric Acid.

Benzoic aldehyde, toluene, cinnamic acid, quinic acid, and phenyl-propionic acid when ingested, are also excreted as hippuric acid. Substituted benzoic acids appear in the urine as substituted hippuric acids. (See salicyluric acid, page 388.)

The quantity of hippuric acid excreted in normal human urine is stated to range from 5 to 60 grains (0·3 to 3·8 grammes) in twenty-four hours, but an increase results from a vegetable diet. This has been particularly noticed after eating plums, pears, and cranberries, and the cuticular parts of many plants act similarly. In the urine of diabetic patients, hippuric acid is frequently present in much increased proportion, as also in jaundice and other liver complaints, and it is abundant in the acid urine of persons suffering from all kinds of fevers.

Hippuric acid replaces uric acid in the urine of herbivorous animals, which are stated to contain it to the extent of about 2 per cent. ; its origin being doubtless in bodies of the aromatic series existent in the food. Hippuric acid is also found in the excrement of the lower animals, except that of birds, which contains the allied substance ornithuric acid, having the constitution of a dibenzoyl-diamidovaleric acid :—

$$\left.\begin{array}{l} C_6H_5.CO.NH \\ C_6H_5.CO.HH \end{array}\right\} :C_4H_7.COOH$$

On boiling ornithuric acid with hydrochloric acid, it almost immediately parts with one benzoyl-group and yields benzoyl-ornithine, which on further boiling splits up into benzoic acid and diamido-valeric acid or ornithine, $(NH_2)_2C_4H_7.COOH$, a base of strong alkaline reaction and of caustic taste.

When boiled for a time (half an hour) with dilute nitric, hydrochloric, or oxalic acid (or more rapidly if strong hydrochloric acid be used), hippuric acid undergoes hydrolysis, the liquid on cooling depositing benzoic acid, while a salt of glycocine remains in solution :—$C_9H_9NO_3+H_2O=C_7H_6O_2+C_2H_5NO_2$. This reaction is employed in practice for the preparation of glycocine (see page 206). A similar reaction takes place spontaneously in urine containing hippuric acid, under the influence of ferments. Hence only perfectly fresh urine will yield hippuric acid. If the urine be alkaline, as is usually the case with that of herbivorous mammals, the glycocine first produced is further changed.

A. E. Garrod has observed (*Lancet*, April 21, 1882) that contact of solutions of alkaline hippurates with uric acid causes its disappearance, so that on adding hydrochloric acid after some hours uric acid can no longer be detected either by the microscope or by the murexide test. Acting on this observation, Garrod has employed sodium benzoate and hippurate with great advantage in cases of gout, gravel, and calculus ; preferring, however, the corresponding potassium or lithium salts in cases where it was desired to increase the excretion of urine.

Hippuric acid is distinguished from benzoic and salicylic acids by its crystalline form (figs. 24 and 25) ; by charring when heated with strong sulphuric acid ; by giving off ammonia on ignition

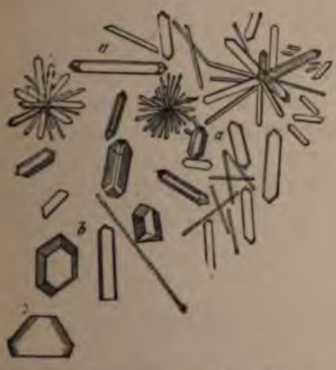

Fig. 24.—HIPPURIC ACID (after Frey)—*a*, *a*, Prisms ; *b*, Crystals formed by slow evaporation.

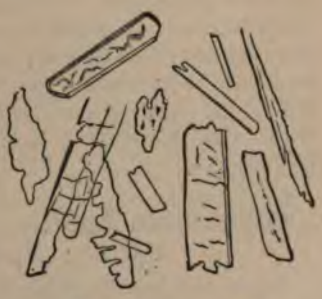

Fig. 25.—BENZOIC ACID.

with soda-lime ; and by not being dissolved on agitating its solution with chloroform or petroleum-spirit. When heated, benzoic acid sublimes unchanged ; but hippuric acid gives red oily drops, and evolves an odour of hydrocyanic acid. When precipitated by hydrochloric acid, hippuric acid separates immediately in needles, whereas benzoic acid forms scales. Hippuric acid is less soluble than benzoic acid in ether. From the neutral solution of a hippurate a neutral solution of ferric chloride throws down ferric hippurate as a cream-coloured precipitate, whereas the precipitate yielded by a benzoate with ferric chloride is reddish-brown.

The preparation, characters, and reactions of hippuric acid have been fully described in Part i. page 23 *et seq.* A method, not previously given, for the determination of hippuric acid in urine is to treat from 1000 to 1200 c.c. of the sample with a slight excess of strong baryta-water. The filtered liquid is treated with dilute sulphuric acid till exactly neutral to litmus, decanted or filtered from the precipitated barium sulphate, and evaporated to a syrup on the water-bath. The residue, which should be exactly neutral, is treated while still hot with 150 to 200 c.c. of absolute alcohol, and thoroughly agitated. Barium succinate, sodium chloride, and other compounds are thus precipitated. The liquid is decanted, the alcohol evaporated, and the syrupy residue treated while still hot with hydrochloric acid. The liberated hippuric acid is extracted by repeated agitations with ether (100 to 150 c.c.), the separated ether distilled off, the residue diluted with water, and heated to boiling with a little milk of lime. The liquid is filtered, concentrated, and treated with excess of hydrochloric acid, when hippuric acid separates in fine crystals, which can be obtained colourless by treatment with purified animal charcoal.

SALICYLURIC ACID, $C_9H_8(OH)NO_3$, has the constitution of a hydroxy-hippuric acid. It occurs in the urine after administration of salicylic acid, which has the constitution of ortho-hydroxybenzoic acid, and may be detected therein by the bluish-violet coloration produced on adding dilute ferric chloride. Salicyluric acid is more soluble than hippuric acid. On boiling with hydrochloric acid, it is split up into salicylic acid and glycocine.

Glycuronic Acid.

$C_6H_{10}O_7$; *i.e.*, $COH(CH.OH)_4.COOH$

Glycuronic acid doubtless has its origin in the dextrose of the body, to which compound it is closely related.[1] It was first

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[1] The relation between glycuronic acid and bodies of the sugar-group is shown by the following constitutional formulæ ;—

Dextrose, ...	$CH_2(OH).(CH.OH)_4.CO.H$
Gluconic acid, . . .	$CH_2(OH).(CH.OH)_4.CO.OH$
Saccharic acid, . . .	$CO(OH).(CH.OH)_4.CO.OH$
Glycuronic acid, . . .	$CO(OH).(CH.OH)_4.CO.H$
Gulonic acid, . . .	$CO(OH).(CH.OH)_4.CH_2.OH$
Gulose, ...	$CO(H).(CH.OH)_4.CH_2.OH$

obtained in the conjugated form of campho-glycuronic acid in the urine of dogs to which camphor has been administered, and subsequently as uro-chloralic acid after the administration of chloral. Glycuronic acid is remarkable for its tendency to form ethereal or glucosidal compounds when appropriate substances are introduced into the body. Traces of such compounds probably occur normally in urine, especially indoxyl- and skatoxyl-glycuronic acids; in addition to the combination with urea, having probably the constitution of uro-glycuronic acid, which appears to be the ordinary form in which glycuronic acid exists in urine.

Baeyer (*Annalen*, clv. 257) has shown that euxanthic acid, which exists in combination with magnesia in the "purrée" or "Indian yellow" of commerce,[1] is decomposed on boiling with hydrochloric acid or dilute sulphuric acid, with formation of euxanthone and an acid which has been shown by Spiegel

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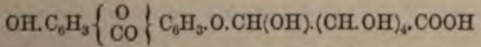
[1] *Piuri* or *Purrée*, now used as a pigment under the name of "Indian yellow," is obtained in Bengal from the urine of cows which are fed exclusively on the leaves of the mango tree and water. The urine is heated, and the precipitate separated and dried. Analyses of very pure specimens of purrée by C. Graebe (*Annalen*, ccliv. 265) showed: euxanthic acid, 51; silica and alumina, 1·5; magnesia, 4·2; lime, 3·4; and water and volatile substances, 39 per cent. The analyses of Stenhouse and Erdmann show much less lime. Urea, uric acid, and hippuric acid have also been found in purrée. The poorer qualities contain considerable quantities of euxanthone, partly free and partly in combination. For the isolation of the euxanthic acid and euxanthone, and the assay of purrée, the colouring matter should be triturated with dilute hydrochloric acid until the whole has assumed the bright yellow colour of euxanthic acid. The residue is then well washed with cold water to remove the salts, and the euxanthic acid extracted from the residue by ammonium carbonate solution. It is precipitated from the filtrate by hydrochloric acid, and purified by crystallisation from alcohol. The euxanthone, left undissolved by the ammonium carbonate, is treated with caustic soda, the solution precipitated with an acid, and the precipitated euxanthone shaken out with ether or filtered off and dried at 100°.

Euxanthic acid has the constitution:—



$$OH.C_6H_3\left\{\begin{matrix}O\\CO\end{matrix}\right\}C_6H_3.O.CH(OH).(CH.OH)_4.COOH$$

It forms pale yellow needles, which melt at 156°–158°. It has a sweet taste and bitter after-taste, is but slightly soluble in cold water, very sparingly in ether, but readily in boiling alcohol. Alkalies colour the solution deep yellow. Euxanthic acid does not reduce Fehling's solution, nor form a compound with phenyl-hydrazine.

Euxanthone is a neutral substance, crystallising in pale yellow needles, soluble in alkalies but not in dilute acids. It forms no compound with phenyl-hydrazine.

(*Ber.*, xv. 1965) to be identical with glycuronic acid, $C_{19}H_{18}O_{11} = C_{13}H_8O_4 + C_6H_{10}O_7$. In fact purrée is the best material for the preparation of glycuronic acid, which can be obtained on the small scale by the following process:—The artists' water-colour known as "Indian yellow" is ground up with sand, and then treated with dilute hydrochloric acid, which dissolves out calcium and magnesium salts, &c. The residue is washed with water and treated with a solution of ammonium carbonate, which dissolves the euxanthic acid, leaving euxanthone and sand undissolved. From the filtered liquid the euxanthic acid is precipitated by dilute hydrochloric acid, washed with cold water, and then heated with water in a closed soda-water bottle to 125° C. for three or four hours. The requisite temperature can be conveniently obtained by immersing the bottle in a bath of molten paraffin wax (candles). From the cooled product the euxanthone is dissolved by agitation with ether, and the glycuronic anhydride crystallised from the concentrated aqueous liquid.

Glycuronic acid is a syrupy liquid, miscible with water or alcohol. When the aqueous solution is boiled, evaporated, or even allowed to stand at the ordinary temperature, the acid loses the elements of water and yields the anhydride or lactone.

GLYCURONIC ANHYDRIDE, $C_6H_8O_6$, forms monoclinic tables or needles, having a sweet taste, and melting at about 160° when heat is gradually applied, or at 170°–180° when heated rapidly. The anhydride is insoluble in alcohol, but dissolves readily in water to form a dextro-rotatory solution. $[\alpha_D] = 19{\cdot}25°$. The solution prevents the precipitation of cupric solutions by alkalies, and powerfully reduces hot Fehling's solution, the cupric oxide reducing power being 98·8, compared with glucose as 100.

Glycuronic acid itself is dextro-rotatory ($[\alpha]_D = +35°$), but many of its compounds are lævo-rotatory.[1] It reduces Fehling's solution on heating, and precipitates the metals from hot alkaline solutions of silver, mercury, and bismuth.

On oxidation, glycuronic acid yields camphoric and formic acids. By treatment with bromine it yields saccharic acid, $C_6H_{10}O_8$, a reaction which indicates the presence of an aldehyde-group and the close relation between glycuronic acid and dextrose. Saccharic acid can again be reduced to glycuronic acid by treatment with sodium amalgam, further treatment yielding gulonic acid, $C_6H_{12}O_7$, a body which does not reduce Fehling's solution.

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[1] After taking chloral hydrate the urine contains trichlorethyl-glycuronic acid ("uro-chloralic acid"), a lævo-rotatory body which is decomposed into trichlorethyl alcohol and dextro-rotatory glycuronic acid.

(Fischer and Piloty, *Ber.*, xxiv. 521; abst. *Jour. Chem. Soc.*, 1891, 667.)

When boiled with caustic alkali, glycuronic acid yields oxalic acid as an invariable product. Catechol and protocatechuic acid are also formed if concentrated alkali be employed for the treatment.

Glycuronic acid is distinguished from glucose by not undergoing the alcoholic fermentation when treated with yeast. On the other hand, when fermented in presence of cheese and chalk it yields lactic and acetic acids.

Glycuronic acid forms a potassium salt which crystallises in needles. The sodium salt is similar. The zinc, cadmium, copper, silver, and calcium salts are uncrystallisable. The barium salt is amorphous and soluble in water. It is the compound employed for the isolation of glycuronic acid from urine.

With phenyl-hydrazine, glycuronic acid forms a yellow crystalline compound melting at 114° to 115° C., but under modified conditions an amorphous, brownish-yellow body, melting at 150° C., is produced. According to J. A. Hirschl, normal urine yields this compound.

For the actual isolation of glycuronic acid from urine a large quantity of the excretion is required. The method has been described by H. H. Ashdown (*Brit. Med. Jour.*, 1890, i. 169, and *Pharm. Jour.*, [3], xx. 607).

On distillation with hydrochloric acid, glycuronic acid is decomposed with formation of furfuraldehyde, carbon dioxide, and water. Glycuronic anhydride and urochloralic acid undergo a similar decomposition, and a trace of furfurol is also obtainable by similarly treating normal urine.

F. Mann and B. Tollens (*Chem. Centr.*, 1894, ii. 83) have proposed to utilise this reaction for the estimation of glycuronic acid and its derivatives. The carbon dioxide obtained on distilling glycuronic acid with hydrochloric acid amounted to 26·5 per cent., whereas the yield from dextrose or lævulose was not more than 1 per cent. The furfurol yielded by glycuronic anhydride under the same treatment was 15·23 per cent. of the weight taken. Those natural compounds which readily yield glycuronic acid on treatment with dilute acids give furfurol on distillation with hydrochloric acid, and the proportion obtained is a measure of the glycuronic acid which may be separated from such compounds. Thus euxanthic acid yielded 6·16 to 7·17 per cent. of furfurol; urochloralic acid, 9·88 to 10·30 per cent.; and potassium urobutylchloralate, 9·50 per cent.

Glycuronic acid occurs in the urine to a very notable extent after the administration of morphine, chloroform, chloral, butyl-chloral, nitrobenzene, camphor, curare, and certain other drugs. It was undoubtedly mistaken for glucose by the older observers. In one case recorded by Ashdown large amounts of glycuronic acid occurred in the urine of a healthy young man, whose excretion was not abnormal either in volume or density.[1]

ACIDS OF BILE.

The bile contains certain conjugated acids which are strictly peculiar to that secretion. They occur as sodium salts,[2] and are not found in the pancreatic juice, or in other normal animal secretions.

Human bile is a reddish, reddish-brown, or dirty green liquid, having an odour like that of musk, a very bitter taste, and a faintly alkaline reaction. The specific gravity averages about 1·020. In its original condition, bile rapidly putrefies, but if the secretion be diluted, acidulated with acetic acid, and filtered from the precipitated mucin, &c., it may be readily preserved.

Bile is a secretion of a very variable character, and its collection in a normal state is attended with peculiar difficulties. The mount secreted daily by a man is stated to average 50 ounces, nd by a woman from 42 to 44 ounces. The following analysis hows the general composition of the solids of human bile :—

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[1] Glycuronic acid must not be confounded with *glycosuric acid*, a body extracted by J. Marshall (*Arch. Pharm.*, [3], xxv. 593, and *Jour. Chem. Soc.*, lii. 1047) from pathological urine by a process based on the insolubility of the lead salt in alcohol of 45 per cent. Glycosuric acid crystallises in opaque tetragonal prisms, which melt at 140°, are readily soluble in water, alcohol, and ether, less readily in chloroform, and insoluble in benzene, toluene, and petroleum spirit. Glycosuric acid contains no nitrogen, is readily and completely absorbed by animal charcoal, and appears to be a phenolic derivative. It reduces Fehling's solution more powerfully than glucose, and also reduces silver nitrate; but not bismuth compounds or alkaline solutions of picric acid. On evaporating the ethereal solution of glycosuric acid at 60° the liquid takes a wine-red colour, which is imparted to the crystals which separate, but these form a colourless solution in water.

A. Geyger (*Pharm. Zeit.*, 1892, page 1488) extracted glycosuric acid from a diabetic urine by acidulating it with sulphuric acid and agitating with ether. The ether left on evaporation a crystalline substance, melting at 143° C., which proved to be the acid in question. He suggests that diabetic urine should always be examined in this manner.

[2] In the bile of fresh-water fish the bile-acids exist as sodium salts, but in the bile of salt-water fish the potassium salts of these acids are said to predominate.

Sodium glycocholate and taurocholate, . . .	9·14	per cent.
Cholesterin, lecithin, fat, and traces of soaps, .	1·18	,,
Bile-pigments; bilirubin, biliverdin, &c., . . Mucin,[1]	3·98	,,
Inorganic salts; chiefly sodium chloride, and iron, calcium, and magnesium phosphates, . .	0·78	,,
Total solids, ...	14·08	,,
Water, ...	85·92	,,
	100·00	

The proportion of solids shown in the above analysis is some-hat above the average. The general range of solids is from 8 to 2 per cent., being highest after eating; but the bile obtained irect from the liver is not strictly identical with that contained n the gall-bladder.

A complex and concentrated solution such as bile is very pt to form deposits under abnormal conditions. Hence arise he well-known concretions called biliary calculi and gall-tones.[2]

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[1] The mucin of human bile appears to be true mucin, and not a nucleo-lbumin as in ox-bile.

[2] Biliary Calculi.—Under this denomination are comprised all those oncretions which are formed in the bile. They are found in all parts of the iliary apparatus, occurring most frequently in the gall-bladder or gall-ducts, ut sometimes in the intestinal canal. Their size varies from very small ranules to (occasionally) that of a pigeon's egg. The form is generally oval, ut when several calculi occur together in the gall-bladder, facets are gener-lly formed by their mutual attrition. The colour of biliary calculi ranges rom nearly white to yellow, brown, and dark green. Gall-stones are generally rittle, and can be readily reduced to a powder having a greasy feel.

Gall-stones usually contain cholesterin as their leading constituent, calcium arbonate and bile-pigments being also present in very variable proportion. 'ats, silica, uric acid, and compounds of iron, zinc, copper, and manganese ave been observed as occasional constituents. Sometimes the bile-pigments reponderate, occasionally amounting to 60 per cent. of the calculus. Besides ilirubin and biliverdin, there have been found in gall-stones :—iliprasin, $C_{16}H_{22}N_2O_6$, bilifuscin, $C_{16}H_{10}N_2O_4$ (?), bilicyanin, ilihumin, &c. The bilirubin exists as a calcium salt, $Ca(C_{16}H_{17}N_2O_3)_2$, hich circumstance prevents the solution of the colouring matter in chloro-rm unless the stone be previously treated with acid. On boiling powdered all-stones with alcohol or ether, cholesterin is almost the only constituent issolved. Dilute hydrochloric acid will subsequently dissolve the calcium, A very complete analysis of human bile has been published by T. Fairley (*Pharm. Jour.*, [3], xxi. 316). For analyses of ox-bile see F. Emich (abst. *Jour. Chem. Soc.*, xlii. 1218).

The two chief acids of bile are glycocholic acid and taurocholic acid. The former of these is the more abundant in human and ox-bile, in the proportion of fully three to one; but is replaced by taurocholic acid in the bile of the dog and carnivorous animals generally. Other bile-acids of less frequent occurrence and abundance are also met with (page 396).

For the preparation of the mixed sodium salts of the bile-acids, ox-bile should be mixed with washed sand, and evaporated at 100° till the residue can be powdered. The product is then extracted with boiling absolute alcohol, which dissolves the salts of the bile-acids, while leaving pigment, mucin, and a portion of the inorganic salts undissolved. The green alcoholic solution is filtered and boiled with animal charcoal till colourless,[1] when it is again filtered, the filtrate evaporated to a syrup, the residue taken up in a minimum quantity of absolute alcohol, and ether added until a permanent turbidity is produced. On standing for a few hours, the mixed sodium salts of glycocholic and taurocholic acids will be deposited as a white, semi-crystalline mass known as "Plattner's crystals," which should be pressed between blotting-paper and dried.

From the sodium salts prepared as above, free glycocholic acid may be readily obtained by dissolving the crystals in a little water, adding ether, and then dilute sulphuric acid as long as a precipitate is produced. On stirring, glycocholic acid separates as a crystalline mass of shining needles, while the very soluble taurocholic acid remains in solution.

Both glycocholic and taurocholic acid readily undergo hydrolysis under the influence of dilute acids or alkalies. In each case one of the products of the reaction is cholalic acid. In the case of glycocholic acid, the second product is glycocine (page 206), while taurocholic acid yields taurin (page 230). The following equations express the reactions:—

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whether existing as carbonate or as the bilirubin compound, and chloroform will then dissolve the bilirubin and bilifuscin. Subsequent boiling with alcohol will dissolve biliverdin and biliprasin, while bilihumin remains insoluble.

Biliary calculi are usually saturated with bile, which has desiccated after removal from the organism. The nucleus generally consists of mucus.

[1] An alternative plan is to mix the original bile into a paste with animal charcoal, dry the mixture at 100°, and exhaust it with boiling absolute alcohol.

$$a.\quad \underset{\text{Glycocholic acid.}}{C_{26}H_{43}NO_6} + H_2O = \underset{\text{Cholalic acid.}}{C_{24}H_{40}O_5} + \underset{\text{Glycocine.}}{C_2H_5NO_2}$$

$$b.\quad \underset{\text{Taurocholic acid.}}{C_{26}H_{45}NSO_7} + H_2O = \underset{\text{Cholalic acid.}}{C_{24}H_{40}O_5} + \underset{\text{Taurine.}}{C_2H_7NSO_3}$$

These changes occur naturally in the intestines. In a state of health, by far the larger proportion of the products is re-absorbed, and passes back to the liver.

The rarer or less-known bile-acids undergo similar changes. Thus:—

GLYCOFELLIC ACID, from human bile, on hydrolysis yields fellic acid, $C_{23}H_{40}O_4$.

HYO-GLYCOCHOLIC ACID and HYO-TAUROCHOLIC ACID, from pig's bile, yield hyo-cholalic acid, $C_{25}H_{40}O_5$.

CHENO-GLYCOCHOLIC ACID from goose-bile yields cheno-cholalic acid, $C_{27}H_{44}O_4$.

Glycocholic Acid. $C_{26}H_{43}NO_6$.

This acid was first described by Gmelin, in 1826, under the name of cholic acid.[1] It occurs as a sodium salt in human and ox-bile to the extent of three to five per cent., together with more or less of the analogous taurocholic acid. The bile of the herbivora generally contains glycocholic acid, but that of the carnivora contains taurocholic acid with mere traces of glycocholic acid.

Glycocholic acid may be readily prepared by the process described on page 394. By pressing the crystals, and recrystallising the acid from hot water, it is obtained perfectly pure.

Marshall prepares glycocholic acid by treating fresh bile with a little hydrochloric acid, and filtering from the precipitate of mucin, &c. 100 measures of the filtrate are then treated with 5 measures of hydrochloric acid and 30 measures of ether, and the mixture shaken and allowed to stand for some hours.[2] The crystals of glycocholic acid which form are then filtered off, washed with water containing ether and hydrochloric acid, dried in the air, and recrystallised from hot water.

Glycocholic acid forms fine glistening needles, which taste at first sweet and afterwards bitter. It melts at 132° to 134° C.

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[1] The name cholic acid has also been applied to the acid produced by the hydrolysis of glycocholic acid. Owing to the confusion thus occasioned, it is better to abandon the term entirely, calling the conjugated acid glycocholic acid, and the product of its hydrolysis cholalic acid.

[2] F. Emich (abst. *Jour. Chem. Soc.*, xlii. 1218) recommends the use of benzene instead of ether in this process, and states that bile which gives no precipitate with ether readily gives crystals when benzene is employed.

(Emich), is soluble in about 3000 parts of cold or 120 parts of boiling water, and is very soluble in alcohol; but is very slightly soluble in ether, and practically insoluble in chloroform and benzene. Glycocholic acid forms salts which are extremely soluble both in water and in alcohol, but very slightly soluble or insoluble in ether. An alcoholic solution of the free acid has an optical activity of $[a]_D = +29{\cdot}0°$; but the specific rotation of the salts is $+25{\cdot}7°$.

Sodium glycocholate, $NaC_{26}H_{42}NO_6$, forms stellate needles. Potassium glycocholate occurs in the bile of certain fishes.

When dissolved in warm concentrated sulphuric acid (or, according to Strecker, by simply heating above 100°), glycocholic acid loses the elements of water, and is converted into glycocholonic acid, $C_{26}H_{41}NO_5$, a body forming an insoluble barium salt but possessing nearly the same optical activity as the parent acid.

As stated on page 394, when boiled with dilute acids or alkalies, glycocholic acid undergoes hydrolysis with formation of cholic acid and glycocine. The reaction is analogous to the formation of benzoic acid and glycocine from hippuric acid.[1]

Taurocholic Acid. $C_{25}H_{45}NSO_7$.

This acid is a constituent of the bile of all carnivora, and exists in human bile together with glycocholic acid. The preparation of pure taurocholic acid from human or ox-bile is difficult, since the portion of the glycocholic acid which remains in solution with the more readily soluble taurocholic acid is very troublesome to separate therefrom. Hence it is preferable to prepare taurocholic acid from dog's bile, which should be treated by the process described on page 394 for the preparation of "Plattner's crystals." The sodium salt is dissolved in water, and the taurocholic acid precipitated as a lead salt by addition of ammonia and basic acetate of lead. The precipitate is filtered off, washed, suspended in alcohol, and decomposed by sulphuretted hydrogen. The filtered liquid is concentrated and treated with excess of ether, when taurocholic acid is precipitated as a syrupy mass, which may become partly crystalline on standing.

The amount of taurocholic acid present in bile may be determined without isolating it by calculation from the amount of sulphur contained in the alcoholic extract of the bile, since no other sulphuretted substance passes into the alcoholic solution. For this purpose, the dried alcoholic extract from a known quantity of bile is evaporated to dryness on a water-bath with fuming nitric acid, by which treatment the sulphur is converted into sulphuric acid. The residue is taken up with water, and the solution precipitated with barium chloride. One part of $BaSO_4$ corresponds to 2·16 parts of taurocholic acid.

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[1] According to F. Emich, when a saturated aqueous solution of glycocholic acid is boiled for many hours, about 22 per cent. is converted into paraglycocholic acid, an intensely bitter substance melting at 183°, and nearly insoluble in water. It seems probable that under the treatment employed the glycocholic acid suffers hydrolysis, with formation of cholalic acid or one of its decomposition-products, which is the substance obtained by Emich.

Taurocholic acid forms deliquescent silky needles, very soluble in water and in alcohol, but insoluble in ether. The same ready solubility characterises the salts of taurocholic acid, except the precipitate produced by basic lead acetate in presence of ammonia, which is insoluble in water or in alcohol.

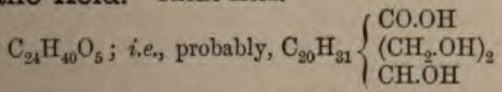
Taurocholic acid and its salts are dextro-rotatory, the value of $[\alpha]_D$ for the solution of the sodium salt in alcohol being $+24{\cdot}5°$.

By boiling with dilute acids or alkalies, taurocholic acid is hydrolysed with formation of cholalic acid and taurine (page 395). The same decomposition occurs on merely boiling an aqueous solution of taurocholic acid, and takes place naturally in the intestines. This behaviour accounts for the absence of unchanged taurocholic acid from the urine, in which it is represented by taurine and taurocarbamic acid.

Taurocholic acid possesses the power of completely precipitating albumins and globulins from their solutions, but it does not precipitate peptones. It is stated to possess powerful antiseptic properties.

Sodium taurocholate has been recommended in cases of gout, obesity, and dyspepsia (*Lancet*, April 25th, 1885, and *Pharm. Jour.*, [3], xv. 948). The salt is prepared by exhausting dried ox-gall (a very bad source) with alcohol, and adding ether gradually till a permanent precipitate is produced, when the sodium glycocholate is deposited in crystals and the taurocholate separates in resinous drops on the sides of the vessel. The commercial product is a buff-coloured powder of peculiar taste, producing heartburn.

Cholalic Acid. Cholic Acid.



$$C_{24}H_{40}O_5\text{; } \textit{i.e.}\text{, probably, } C_{20}H_{31}\begin{cases} CO.OH \\ (CH_2.OH)_2 \\ CH.OH \end{cases}$$

Cholalic acid is the acid product of the hydrolysis both of glycocholic acid and taurocholic acid, the former yielding glycocine and the latter taurine as the basic product of the decomposition (page 395).

Cholalic acid occurs in the small and large intestines as a product of the decomposition of the bile-acids. It is also present in the fæces of men and the lower animals, and, under abnormal conditions, in urine.

For the preparation of cholalic acid, ox-bile should be boiled for twenty-four hours, in a flask fitted with a reflux condenser, with as much caustic baryta as it will take into solution. The liquid is filtered while still hot, and the filtrate concentrated until it yields a copious crop of crystals of barium cholalate. The salt is recrystallised from boiling water, decomposed by hydrochloric acid, and the free cholalic acid crystallised from a small volume of boiling alcohol. Or the acid may be dissolved in caustic soda containing a little ether, and the solution acidulated with hydrochloric acid. The crystals which form after a time are separated and treated with ether, which is poured off after half an hour, and the residue dissolved in boiling alcohol. Water is gradually added to this solution till a permanent precipitate appears, when cholic acid will crystallise out on cooling.[1]

As thus prepared from ox-bile, cholic acid forms rhombic octahedra or tetrahedra which, according to Strecker (*Annalen*, lxvii. 1), contain 2½ aqua.[2] The crystals are very sparingly soluble in water, requiring 750 parts even of the boiling solvent;

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[1] The same method may be employed for the estimation of the cholalic acid obtainable from bile, but for this purpose Lassar-Cohn (*Ber.*, xxvi. 146) recommends the following process:—Twenty c.c. measure of the bile is mixed with 2 grammes of caustic soda, the liquid boiled for twenty-four hours, saturated with carbon dioxide, and evaporated to dryness at 100°. The residue is boiled with nearly absolute alcohol, until free from the salts of organic acids which are only sparingly soluble in water, and the solution, after dilution with four measures of water, is precipitated by 0·5 gramme of barium chloride in dilute solution. The filtered liquid is acidified with hydrochloric acid, and shaken with ether. This, in presence of the alcohol, readily extracts the cholalic acid, which is obtained on evaporating the ethereo-alcoholic solution. Lassar-Cohn obtained the following percentage of acids from a sample of ox-bile treated in the above manner:—Cholalic acid, 4·790; choleic acid, 0·085; myristic acid, 0·004; stearic and palmitic acids, 0·146: resinous acids, 0·120; and loss, 0·050 per cent.

[2] According to Mylius (*Ber.*, xix. 369) cholic acid crystallised from alcohol contains $C_{24}H_{40}O_5 + C_2H_6O$, and does not contain 2½ H_2O, as supposed by Strecker. Cholic acid crystallises similarly from methyl alcohol or acetone combined with one molecule of the solvent. From aqueous solutions it separates either in minute anhydrous crystals or in rhombic plates with 1 aqua.

Mylius states that bile which has been allowed to putrefy for some time does not yield cholic acid on saponification, but a mixture of choleic acid and desoxycholic acid, $C_{24}H_{40}O_4$.

but are readily dissolved by alcohol. An amorphous form of the acid is said to be obtainable by evaporating its solution to dryness. It is described as more soluble than the crystals, and separating in anhydrous prisms from its solution in ether. When sodium cholalate is decomposed under ether by adding hydrochloric acid, the cholalic acid separates in rhombic plates containing one molecule of water.

Cholalic acid is dextro-rotatory, the value of $[\alpha]_D$ for the anhydrous acid being $+50^\circ$, while that for the crystallised form containing $2\frac{1}{2}$ aqua is said to be only $+35^\circ$. Hoppe-Seyler found the rotation of cholalic acid in alcoholic solutions of the sodium salt to be $[\alpha]_D = +31{\cdot}4^\circ$.

Monacetyl- and diacetyl-derivatives of cholalic acid have been obtained.

Iodocholic Acid.—Cholalic acid forms a curious compound with iodine, to which the formula $(C_{20}H_{40}O_5I)_4,HI + xH_2O$ is ascribed. The potassium salt of this body is best obtained by adding a concentrated aqueous solution of 1 gramme of potassium iodide to a solution of 2 grammes of cholalic acid and 0·8 gramme of iodine in 40 c.c. of alcohol. The solution is gradually diluted with water until the compound separates out as a bright blue precipitate. This, when collected and washed with water, forms a bronze-coloured mass. When suspended in 500 c.c. of water, the product forms an indigo-blue liquid which on heating becomes yellow with separation of cholalic acid. The same decomposition occurs by excessive dilution in the cold, the solution being then found to contain free iodine. Sulphurous acid and other reducing agents decompose the blue liquid with separation of cholalic acid, and the same decomposition is produced by adding a few drops of caustic soda, but is restored on the addition of acid. Free iodocholic acid is obtained on adding a small quantity of hydriodic acid to the brown solution of iodine and cholalic acid in alcohol. The liquid immediately becomes blue, and the product may be isolated in a manner similar to the potassium salt which it closely resembles. When iodocholic acid is dried in a vacuum, a dark, lustrous, crystalline powder is obtained which dissolves in ether containing alcohol to a yellow solution. This on evaporation leaves anhydrous iodocholic acid as an amorphous yellow substance which becomes blue in presence of water.

The production of a deep blue coloration on adding iodised potassium iodide to its solution is a valuable and characteristic test for cholalic acid, especially as the reaction is not produced by choleic acid, by the products of the decomposition of cholalic acid, nor by the conjugated acids of bile.

Barium cholalate forms fine silky needles (often radiated), very soluble in boiling water and in alcohol.

The sodium and barium salts of the cholalic acid isolated from human bile are less soluble than the corresponding compounds prepared from ox-bile. The difference has been attributed to the non-identity of the cholalic acids from the two sources, but it appears to be really due to contamination of the cholalic acid from human bile with a small quantity of the analogous fellic acid. The cholalic acids from the bile of several other animals also exhibit certain differences, which in some cases at least are due to the presence of associates of ordinary cholic acid. Thus:—

CHOLEIC ACID, $C_{25}H_{42}O_4$, is obtained in small quantity, together with cholic acid, when the latter is prepared from ox-bile. It is soluble at 20° in 22,000 parts of water, in 750 of ether, in 14 parts of absolute alcohol, and in 25 parts of alcohol of 75 per cent. Barium choleate dissolves in 1200 parts of cold water, the solubility rapidly increasing with the temperature. Choleic acid is dextro-rotatory, the value of $[a]_D$ at 20° C., in a 6 per cent. solution in alcohol of 0·811 specific gravity, being $+8·1°$.

DESOXYCHOLIC ACID, $C_{24}H_{40}O_4$, was obtained, together with choleic acid, from bile which had been allowed to putrefy for some time. Desoxycholic acid differs from cholalic acid in its taste, its ready solubility in alcohol, and in its sparing solubility in acetic acid.

FELLIC ACID, $C_{23}H_{40}O_4$, occurs with cholalic acid in human bile. It has a bitter taste, melts at 120°, and forms very sparingly soluble barium and magnesium salts. Fellic acid gives a red colour but not a violet colour with Pettenköfer's test (C. Schotten, *Zeits. physiol. Chem.*, xi. 268).

HYOCHOLIC ACID, $C_{25}H_{40}O_4$, and CHENOCHOLIC ACID, $C_{27}H_{44}O_4$, are obtained by the hydrolysis of the conjugated acids of the bile of the pig and the goose respectively.

CHOLOIDIC ACID, $C_{24}H_{38}O_4$, and DYSLYSIN, $C_{24}H_{36}O_3$, are products of the dehydration of cholalic acid by boiling for some time with hydrochloric or sulphuric acid, or by exposure to a temperature of 200° C. Dyslysin is an amorphous substance, soluble in a large quantity of ether, and is dissolved by solutions of cholalic acid and its salts. The various modifications of cholalic acid obtainable from different sources are each said to yield their own variety of dyslysin.

The colour-reaction for bile acids known as Pettenköfer's test is described on next page.

The following is a tabular scheme for the separation of bile-acids:—

SCHEME FOR THE SEPARATION OF BILE ACIDS.

<table>
<tr><td colspan="4">Dissolve the sodium salts, precipitated by ether, in water, and precipitate the solution by neutral lead acetate.</td></tr>
<tr><td colspan="2">PRECIPITATE contains the lead salts of cholalic and glycocholic acids. Boil with alcohol, and evaporate the solution to dryness with sodium carbonate. Take up with alcohol, and precipitate the filtered liquid with ether; both acids give crystalline sodium salts. Sodium glycocholate forms six-sided prisms, with a single face having very oblique truncations. Agitate the liquid with dilute sulphuric acid and ether, and filter.</td><td colspan="2">FILTRATE contains taurocholic acid. Add a solution of basic lead acetate and ammonia, wash and convert the precipitate to the sodium salt. Boil the latter for six hours with hot saturated baryta-water. The taurocholic acid is hydrolysed, with the formation of *taurin* and *cholalic acid*. Filter the boiling liquid, and pass carbon dioxide through the filtrate. Filter again, and add hydrochloric acid to the filtrate.</td></tr>
<tr><td>PRECIPITATE may contain *choloidic* and *glycocholic* acids. Choloidic or choleïc acid may be recognised by its characteristic resinous appearance, and by its precipitation as a resin on acidifying a solution of one of its salts.</td><td>FILTRATE may contain *cholalic acid*, which may be recognised by its crystalline form, and by the characters of its barium salt (see page 400).</td><td>PRECIPITATE consists of *cholalic acid* (see page 398).</td><td>FILTRATE. Add sufficient sulphuric acid to precipitate any remaining barium. The excess of sulphuric acid is then removed by addition of lead hydroxide, and any lead which may have dissolved is precipitated by passing sulphuretted hydrogen. The solution is finally evaporated to dryness on the water-bath, and the residue taken up with alcohol. Any undissolved matter is *taurin*.</td></tr>
</table>

When it is merely desired to ascertain the amounts of glycocholic and taurocholic acids in a mixture of their sodium salts, this can be effected by oxidising a weighed quantity of the mixture with fuming nitric acid, and converting the resultant sulphuric acid into barium sulphate, as described on page 397. 100 parts of $BaSO_4$ represent 225·3 parts of sodium taurocholate, and by deducting the amount thus found from that of the mixed sodium salts the weight of sodium glycocholate may be obtained.

PETTENKÖFER'S REACTION FOR BILE-ACIDS.—The most delicate and characteristic reaction of cholalic acid is that known as Pettenköfer's test, but which would be more appropriately termed the furfurol reaction. It depends on the vivid purple coloration produced on treating cholalic acid with strong sulphuric acid and furfurol or any substance (such as sugar) capable of yielding furfurol by reaction with the acid. The reaction is common to all the varieties of cholalic acid, and also to the conjugated forms in which they exist in bile from various sources. As the detection of bile, especially in urine, is often of considerable pathological importance, the reaction has a practical interest.

Pettenköfer's reaction is most simply observed by treating a drop of bile on a porcelain surface with a drop of a solution of cane-sugar and adding a drop of strong sulphuric acid. A bright cherry-red colour will be produced, and, either at once or on gently warming the mixture, will rapidly change to a magnificent purple tint, ultimately becoming bluish. Too high a temperature must be carefully avoided, or the reaction will be obscured by charring of the sugar, and excess of sugar should be avoided for the same reason. Hence it has been proposed to employ furfurol instead of sugar in important cases.

In using furfurol, 1 c.c. of the liquid to be tested, which may be either aqueous or alcoholic, is treated in a test-tube with 1 drop of a solution of furfurol in 1000 parts of water. One c.c. of concentrated sulphuric acid is then added, and the tube immersed in water till the temperature does not exceed 50° to 60° C.

The tendency to char which attends the use of cane-sugar may be avoided by employing glucose in its place. If a little glucose be dissolved in concentrated sulphuric acid, and a few drops of the freshly-made reagent be allowed to fall in the centre of a small pool of urine on a white plate, the play of colours produced in the presence of bile-acids may be observed under very favourable conditions.

It has also been proposed to employ phosphoric acid in place of sulphuric acid, but the substitution is not desirable.

Unfortunately, Pettenköfer's reaction is not peculiar to the bile-acids. Udranszky has enumerated seventy-six organic substances which behave somewhat similarly, but of these only α-naphthol gives the reaction as readily as the bile-acids. A useful confirmation of the reaction is afforded by the absorption-spectrum of the colouring matter. For this purpose the colour should be produced as already described, and the purple liquid diluted with glacial acetic acid or alcohol until the tint is of suitable depth for observation of the spectrum. The colouring matter from bile-acids exhibits four absorption-bands. Of these, the band slightly on the red side of the Fraunhöfer line E, and another about F, are the best defined. Two others may be observed near D. On further dilution of the liquid, these two bands disappear entirely, and that between D and E becomes indistinct, but the most refrangible band still persists. The cherry-red colour produced by albumin, when treated with sulphuric acid and sugar, shows but one absorption-band, between E and F, and does not exhibit the dichroism characteristic of the colouring matter from bile-acids.

The colouring matter formed in Pettenköfer's reaction is soluble in ether.

Pettenköfer's reaction is obscured or actually falsified by proteids, fatty matters, and certain colouring and extractive matters, and hence it is important to remove these before employing the test. In applying the test to urine, purification is usually sufficiently effected by rendering the liquid distinctly but not strongly acid with acetic acid, boiling for a minute or two, and filtering from any mucus, albumin, &c., which may be precipitated.

In applying Pettenköfer's test to urine, it is often desirable to concentrate the liquid previously on the water-bath. A little cane-sugar or glucose is then dissolved in it, and a portion of the cold liquid placed in a test-tube. Strong sulphuric acid is then allowed to run down the side of the tube so as to form a distinct layer below the urinous liquid, when the characteristic purple coloration will be developed at the junction of the two strata if any bile-acids be present. An alternative and very delicate mode of performing the test is to dip a slip of filter-paper in the sweetened urine, and allow it to dry spontaneously. *When dry*, a drop of concentrated sulphuric acid is applied to the paper by means of a glass rod, when if bile-acids be present, even to the extent of 0·03 per cent., in less than half a minute a violet stain will be produced on the paper, which is best viewed by transmitted light.

In certain cases it is an advantage previously to isolate the bile-acids from the urine in an approximately pure condition before applying Pettenköfer's test. For this purpose the largest volume of urine available should be boiled, filtered, and treated with lead acetate and ammonia as long as a precipitate forms. The precipitate is filtered off, washed well, pressed, and boiled with alcohol, which dissolves the lead salts of the bile-acids, leaving the urate, phosphate, &c., insoluble. The alcoholic liquid is filtered boiling hot, and evaporated on the water-bath with a few drops of a solution of sodium carbonate. From the residue the sodium salts of the bile-acids are dissolved by alcohol, and to the solution Pettenköfer's and other tests can be advantageously applied. To detect traces of bile-acids, the alcoholic solution of the sodium salts is concentrated to a few drops, and three or four drops of dilute sulphuric acid (1 : 4) added, together with a minute quantity of cane-sugar or glucose. The liquid is then evaporated at a gentle heat, when the characteristic violet coloration will be produced with as little as 0·0001 gramme of bile-acids.

It must be remembered that while Pettenköfer's reaction is given by all the acids of bile, and by the acid products of their hydrolysis, no similar coloration is produced by taurin or by biliary pigments (see below). Medical men often fall into error on this point, and assume the absence of bile-pigments from urine because the sample gives a negative reaction with Pettenköfer's test. It is a fact that in jaundice the urine contains very little bile-acids, and frequently they are entirely absent, while on the other hand the bile-pigments are conspicuously present in the urine of jaundiced persons.

Bile-Pigments.

Bile contains certain colouring matters which are derived from hæmoglobin[1] and are not chemically related to the bile-acids.

BILIRUBIN, $C_{16}H_{18}N_2O_3$,[2] is the yellow pigment of the bile of man and herbivorous animals. It is troublesome to prepare pure, but is best obtained by extracting powdered human gall-stones with ether, which extracts cholesterin. The residue is boiled with water, and treated with dilute hydrochloric acid to decompose the calcium salt, $Ca(C_{16}H_{17}N_2O_3)_2$, which is the form in which bilirubin occurs in gall-stones. The mass is washed, dried, and extracted with chloroform; the chloroform distilled off, and the residue treated with absolute alcohol. It is then again dissolved in chloroform, and precipitated by absolute alcohol.

Bilirubin is an orange powder, insoluble in water, alcohol, or ether, but soluble with some difficulty in benzene and chloroform. It dissolves in alkalies with orange colour, and is precipitated unchanged if hydrochloric acid be at once added, and may be extracted by agitation with chloroform; but if the alkaline liquid be exposed to the air it gradually absorbs oxygen, and then yields a green precipitate of biliverdin, $C_{16}H_{18}N_2O_4$, when acidulated. If the oxidation be carried further, as by adding fuming nitric acid to an alkaline solution of bilirubin mixed with an equal measure of alcohol, a blue pigment, bilicyanin, is formed; next a violet, which is perhaps a mixture of the red and blue; then a red colouring matter; and lastly a yellow pigment, called by Maly choletelin, and said to have the composition $C_{16}H_{18}N_2O_6$.

The foregoing colour-reactions may be conveniently observed by spreading a drop of bile in a thin film on a porcelain plate, and placing a drop of fuming nitric acid in the centre, when a series of rings will be produced, coloured successively green, blue, violet, red, and yellow. By placing the platinum terminals of a battery of four Grove's cells in some bile, the succession of colour-reactions due to the oxidation of bilirubin will be produced round the anode, and can be observed to great advantage. On reversing the current the colour-changes occur in the opposite order.

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[1] The bile-pigments contain no iron, which, however, exists in the bile in the form of phosphate, and is deposited in comparatively large amount in the liver in cases of pernicious anæmia.

[2] Thudichum has attributed to bilirubin the composition $C_9H_9NO_2$; but the correctness of the formula given in the text has been proved by Raoult's method (*Jour. Chem. Soc.*, lviii. 76).

By the reduction of bilirubin in alkaline solution by means of sodium amalgam, Maly obtained hydrobilirubin, $C_{32}H_{40}N_4O_7$, or $C_{32}H_{36}N_4O_5 + 2H_2O$, said by some observers to be identical with urobilin, the colouring matter of normal urine.[1] Hydrobilirubin is also identical with, or closely related to, stercobilin, the colouring matter of fæces in a state of health, but is absent during an attack of jaundice, when the fæces are slate-coloured.

Biliverdin, $C_{16}H_{18}N_2O_4$, exists in human bile, but is especially characteristic of the bile of herbivorous animals. It is produced with great facility by the oxidation of bilirubin, from which it differs in colour (dark green), its insolubility in chloroform, and its ready solubility in alcohol. It is also soluble in benzene and in carbon disulphide, but is only slightly soluble in ether. If fuming nitric acid be added to an alcoholic solution of biliverdin a bluish-violet coloration is produced, changing to red and finally to yellow with excess of acid.

Detection of Bile-Pigments in Urine.—The colouring matters of bile are not present in normal urine, but in certain diseases (jaundice, &c.) they exist in very appreciable amount. Such urine exhibits a yellowish-green, green, greenish-brown, or almost black colour. *Bilirubin* predominates in bilious urine of a saffron-yellow colour; while *biliverdin* and other oxidation-products are present in greenish urine. Bilious urine gives a yellow froth on agitation, and stains linen and filter-paper yellow.[2]

A variety of tests have been proposed for the detection of bile-pigments in urine, but the following are the most delicate, and answer every purpose:—

Gmelin's Test consists in treating the urine with strong nitric acid and observing the change of colour produced. The reaction is best observed by allowing some of the urine to run gently on to the surface of some fuming nitric acid contained in a test-tube. If bile-pigments be present, a green ring will become apparent at the point of contact, while below this will appear violet, red, and yellow zones, in the order named. The green colour is alone characteristic of bilious urine, since indigogens give rise to blue and red colorations. The urine of patients who have taken potassium iodide also gives a red zone with nitric acid.

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[1] This is denied by MacMunn, who obtained a similar substance by the action of reducing agents on hæmatin and hæmatoporphyrin. The whole subject of the relation between the colouring matters of bile and urine requires re-investigation. A recent interesting research on the subject has been published by A. Jollis (*Pflüger's Archiv.*, 1895, lxi. 623; abst. *Jour. Chem. Soc.*, 1896, ii. 51).

[2] If the dyed filter-paper be treated with a drop of nitric acid, the margin of the spot will become violet or deep blue, while the centre gradually changes to emerald-green.

Various modifications of Gmelin's reaction have been proposed, but they possess no advantage over the above mode of applying the test.

Rosin's test for bile-pigments consists in allowing very dilute iodine solution or bromine-water to flow on to the surface of the urine from a pipette. A grass-green ring is produced at the junction of the two strata.

For the detection of *traces* of bile-pigments, often of great clinical and physiological importance,[1] the urine should be treated with a moderate excess of lime-water, and the excess of lime precipitated as carbonate, by passing carbon dioxide gas or adding seltzer-water, till the liquid no longer exhibits an alkaline reaction to litmus (or preferably to phenol-phthalein). The precipitate is collected on a filter, and treated with fuming nitric acid, when the green and other colours already described will become evident if bile be present. Or the precipitate may be boiled with alcohol acidulated with sulphuric acid, when the supernatant liquid will acquire a grass-green colour, a white deposit of calcium sulphate being simultaneously formed. (See also Hilger, *Arch. d. Pharm.*, ccvi. 385.)

Another reliable test for bile-pigments in urine is to treat 30 c.c. (or 1 oz.) of the sample with about one-third of its bulk of a 20 per cent. solution of zinc acetate,[2] after previously neutralising most of the free acid by sodium carbonate. The voluminous precipitate is filtered off, washed, and treated with a little ammonia. In presence of bile-pigments the ammoniacal liquid is usually fluorescent, and either at once or on standing shows the absorption-spectrum of bilicyanin, characterised by bands on each side of the D line, and a third between *b* and F.

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[1] In two specimens of highly icteric urine, after the occurrence of ammoniacal fermentation Salkowski could detect no bilirubin by Gmelin's test, and extraction yielded no unchanged biliary pigment. He suggests that this decomposition of bilirubin without the formation of any characteristic products was probably the result of the activity of bacteria, and may explain other cases of jaundice, in which the urine, though dark coloured, gave no evidence of the presence of bile-pigments.

[2] Zinc acetate may be readily extemporised by treating lead acetate with zinc sulphate in slight excess, and filtering from the precipitated lead sulphate. Or sodium acetate may be added to a solution of zinc sulphate or chloride, the sodium sulphate or chloride formed simultaneously with the zinc acetate being disregarded.

It must be remembered that some urines which contain when fresh only a small amount of bile-pigment, will, after being exposed to the air for several days, show no bilirubin whatever, urobilin having taken its place.[1] The source of the urobilin in the fæces is also doubtless the bile-pigment, unaltered bile-pigment never occurring in normal fæces.

Urinary urobilin exhibits a green fluorescence when the urine is rendered ammoniacal, and a few drops of zinc chloride are added. It shows a well-marked absorption-band between the Fraunhöfer lines *b* and F. The coloured products formed from bilirubin by oxidation, with the exception of the final yellow product choletelin, all exhibit a similar fluorescence with ammonia and zinc chloride, and show an absorption-band near F; but less sharply defined than that in the spectrum of urobilin, which, however, they closely resemble.

From some urines pigments can be separated which possess all the characters of the red and brown oxidation-products of bilirubin, while others yield a substance identical with choletelin. Hence the colouring matters of normal urine, which may be termed physiological urobilins, are oxidation-products of bilirubin, while pathological urobilins are reduction-products of the same substance. Pathological urobilins are produced in some cases from bilirubin, but can be formed from blood-pigment directly after extravasation of blood.

LACTIC ACIDS.

The lactic acids have the constitution of hydroxypropionic acids.[2] Two *chemical* isomers of such constitution are possible, according as they are derived from ethidene (ethylidene) or from ethylene. Ordinary lactic acid is the former of these isomers, while the second, sometimes called hydracrylic acid, is obtainable by synthetical means. The optically active modifications of lactic acid, of which one occurs in the juice of flesh, and is hence called sarcolactic acid, are physical isomers of ordinary lactic acid (page 419). The following formulæ show the constitution of the two chemical modifications of lactic acid:—

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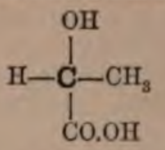
[1] Fresh urine containing but little urobilin often becomes darker on exposure to the air, a change probably due to the formation of urobilin from a substance called by MacMunn *urobilinogen*.

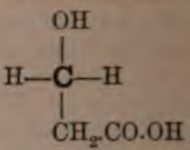
[2] The lactic acids are homologous with glycollic acid, hydroxybutyric acid, and hydroxycaproic or leucic acid.

ETHIDENE LACTIC ACID.
α-Hydroxypropionic Acid.



$$\begin{array}{c} \mathrm{OH} \\ | \\ \mathrm{H—C—CH_3} \\ | \\ \mathrm{CO.OH} \end{array}$$

ETHYLENE LACTIC ACID.
β-Hydroxypropionic Acid.



$$\begin{array}{c} \mathrm{OH} \\ | \\ \mathrm{H—C—H} \\ | \\ \mathrm{CH_2.CO.OH} \end{array}$$

Ethidene Lactic Acid. Inactive Lactic Acid.

Fermentation Lactic Acid. α-Hydroxypropionic Acid.

$C_3H_6O_3$; *i.e.*, $CH_3.CH(OH).COOH$

Lactic acid was first obtained by Scheele, in 1780, from sour milk. It exists ready-formed in both the animal and the vegetable kingdoms.[1] Lactic acid is formed by a peculiar fermentation of carbohydrates such as sugar, gum, starch, mannitol, and particularly of milk-sugar, in the presence of casein or other proteids. Hence lactic acid is contained in sour milk (but not in fresh milk), in sourkrout, pickles, distillery-wash,[2] sour beer, &c. The acid contained in sour tan-liquors and the acid runnings of starch-makers, &c., called by Braconnot nanceic acid, and the so-called thebolactic acid contained in opium are also ordinary lactic acid.

Lactic acid is obtainable by various synthetical processes, including:—The action of nitrous acid on alanine; the oxidation of α-propylene-glycol by nitric acid; the action of alkalies on α-chloro- or bromo-propionic acid; the reaction of aldehyde with hydrocyanic acid, and treatment of the resulting hydroxycyanide with hydrochloric acid[1]; the cautious oxidation of glycol with spongy platinum or dilute nitric acid; &c.

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[1] It is a mistake to suppose that lactic acid is always a product of fermentation, and not a normal product of vegetation. Many seeds contain lactic acid, and Windisch has found it in potatoes. The view that lactic fermentation is due to a specific ferment is contradicted by the investigations of Martmann. Lintner has similarly observed that *Pediococcus acidi lactici* causes a considerable lactic fermentation in malt-worts, and Hayduck has noticed the formation of lactic acid in the spontaneous fermentation of a malt-wort in which no lactic ferments could be detected, and which only contained *sarcina* in bundle form.

[2] Spent distillery wash, technically called pot-ale or burnt ale, contains about 3 per cent. of solid matters, of which about 1 per cent. consists of lactic and other acids, 0·7 of peptones and other nitrogenous matters, and 0·7 per cent. of mineral matters, in which phosphates predominate.

Lactic acid is commonly directed to be prepared by dissolving 2 kilogrammes of cane-sugar and 15 grammes of tartaric acid in 17 litres of boiling water, and allowing the solution to stand for some days. 100 grammes of decaying cheese should then be macerated in 4 litres of sour milk, and added to the sugar solution, together with 1200 grammes of zinc-white. The mixture is kept at 40° to 45° C. for eight or ten days (longer treatment causes the conversion of the lactic acid into butyric acid), when the liquid is boiled, filtered, and the filtrate concentrated to a relatively small bulk. On standing, a mixture of zinc lactate with mannitol crystallises out, which should be separated, pressed strongly, and recrystallised from boiling water. It is then suspended in water, and decomposed by sulphuretted hydrogen, and the filtered liquid concentrated on the water-bath to a syrup. On agitating this with ether, the lactic acid is extracted, the mannitol remaining. The lactic acid is obtained by evaporating the ether.

For the manufacture of lactic acid on a large scale, G. Jacquemin treats malt-wort with pure lactic ferment prepared by Pasteur's method, adds pure sterilised calcium carbonate, and allows the mixture to ferment at 40° to 45° for five or six days. The liquid is then filtered and concentrated, when an inodorous calcium lactate crystallises out.

The formation of lactic acid by the fermentation of glucose may be represented by the equation :—$C_6H_{12}O_6 = 2C_3H_6O_3$, but there is good reason to suppose the reaction to be much less simple than is thus indicated. Numerous organisms are capable of converting sugar into lactic acid, but that known as the lactic ferment (*Bacillus acidum lactici*) has by far the most energetic action. It consists of short thick cells generally united in pairs, and is most active between 35° and 45° C. Access of air is necessary, and nitrogenous food is required. Excess of acid arrests the fermentation, but the action recommences if the liquid be neutralised. 0·05 per cent. of sulphuric acid, or 0·2 per cent. of lactic acid, completely arrests the lactic fermentation. The action is not affected by 2 per cent. of alcohol, but 4 per cent. diminishes it, and 6 per cent. arrests it completely.

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[1] The reaction occurs in two stages, thus :—

a. $CH_3.COH + HCN = CH_3.CH(OH)CN.$

b. $CH_3.CH(OH)CN + HCl + 2H_2O = CH_3.CH(OH).COOH + NH_4Cl.$

In practice, the aldehyde or acetone is dissolved in ether and powdered potassium cyanide added, followed gradually by concentrated hydrochloric acid. This reagent converts the cyanide into lactic acid, the amido-acid being formed in the cold as an intermediate product and converted into the hydroxy-acid by boiling with more dilute acid (hydrochloric or sulphuric).

Lactic acid is formed by the action of alkalies on glucose, and the reaction may be conveniently used for its preparation. Kiliani (*Ber.*, xv. 699) recommends the following method:—500 grammes of cane-sugar are mixed with 250 c.c. of water and 10 c.c. of dilute sulphuric acid (3 parts H_2SO_4 to 4 parts water), and warmed in a large flask to 50° for two hours. The solution is thoroughly cooled, when 400 c.c. of a solution of caustic soda, made by dissolving caustic soda in its own weight of water, is added gradually, the mixture being kept cool throughout. A quantity of sulphuric acid (of the strength specified above) sufficient to exactly neutralise the caustic soda is then added. A crystal of sodium sulphate is put into the solution to assist crystallisation, and the cooled liquid set aside for twenty-four hours. The mass is extracted with alcohol of 93 per cent., the liquid filtered with the aid of a filter-pump, and the lactic acid converted into the zinc salt.

Concentrated lactic acid thus prepared is a colourless, odourless, syrupy liquid of very acid taste. It has a specific gravity of 1·2116 at 15° C., and contains about 75 per cent. of real lactic acid, $C_3H_6O_3$. Absolute lactic acid cannot be obtained, since any attempt to further concentrate the aqueous acid causes the formation of anhydrides. This change occurs when lactic acid is evaporated at the ordinary temperature in dry air. The following table shows the percentage composition of products thus obtained by Wislicenus:—

	Water.	Lactic Acid, $C_3H_6O_3$.	Lactic Anhydride, $C_6H_{10}O_5$.	Lactide, $C_6H_8O_4$.
A. Freshly prepared as above; syrup, . .	15·64	58·80	25·56	...
B. After drying 4 months over sulphuric acid, . . .	4·07	22·43	73·50	—
C. After 13 months; thick syrup insoluble in water, . .	...	...	97·85	2·06
D. After 16 months; treacly syrup, . .	...	...	71·41	28·59
E. After 18 months; thick gummy mass,	...	...	60·77	39·23

It follows that lactic acid is not volatile without decomposition. At 130° it begins to decompose, and at about 145° sparingly soluble lactic anhydride is formed, which at a higher temperature forms lactide and other products.

The lactic acid of the British and United States Pharmacopœias has a specific gravity of 1·21, and is stated to contain about 75 per cent. of lactic acid and 25 per cent. of water. The last statement is incorrect, it having been pointed out by W. L. Scoville that the commercial article contains a notable amount of lactic anhydride. When the acid is diluted with 5 to 10 parts of water, and titrated with caustic alkali, phenol-phthaleïn being preferably used as an indicator, the end-reaction corresponds to the neutralisation of the lactic acid. On standing in the cold, or more rapidly on boiling, the lactic anhydride undergoes more or less hydrolysis, and the previously neutral liquid acquires an acid reaction. By adding caustic alkali in excess, boiling for fifteen to twenty minutes, and titrating back with standard acid the lactic anhydride can be conveniently determined. Scoville finds the amount present to be sometimes as high as 15 per cent.

Lactic anhydride and lactide are nearly insoluble, but are converted by prolonged boiling with water, and readily by solutions of caustic alkalies, into lactic acid. The lactide obtained by heating paralactic acid (dextro-rotatory) yields ordinary inactive lactic acid when treated in this manner.

Lactic acid is miscible in all proportions with water, alcohol, glycerin, and ether. It is but slightly soluble in chloroform, and is insoluble in carbon disulphide and petroleum spirit. Glyceric acid, $C_3H_6O_4$, which resembles lactic acid, is insoluble in ether.

Lactic acid dissolves recently precipitated phosphate of calcium, and is frequently used for that purpose.

Concentrated sulphuric acid mixes with pure lactic acid without blackening it. On heating, a brown colour is developed, and much carbon monoxide evolved, a humus-like body being ultimately left.

On distillation with a large excess of quicklime, lactic acid is converted into carbon dioxide and alcohol. This reaction affords a means of obtaining alcohol from glucose without the intervention of a fermenting organism.

Lactic acid does not reduce Fehling's solution, but rapidly decolorises potassium permanganate, both in acid and in alkaline solutions with production of an odour of aldehyde. Silver lactate is imperfectly reduced on boiling, with production of a blue liquid and a brownish deposit.

Uffelmann's test for free lactic acid is described on page 419.

Lactic acid may be separated from organic acids forming insoluble lead salts by precipitating the solution (previously neutralised if necessary) with neutral lead acetate, either with or without an addition of alcohol. Lead lactate remains in solution, and may be decomposed by sulphuretted hydrogen, when free lactic acid is obtained.

Many admixtures may be separated from lactic acid by saturating the free acid by barium carbonate. When the aqueous solution is evaporated and the residue treated with alcohol, many of the acids whose barium salts are soluble in water remain behind, whereas barium lactate dissolves in alcohol. Free lactic acid may be obtained by cautiously precipitating the solution of barium lactate with dilute sulphuric acid, and filtering.

When purified from all substances except those soluble in alcohol, the aqueous liquid containing free lactic acid may be saturated with oxide of zinc, evaporated to dryness, and the residue digested with alcohol. Lactate of zinc, insoluble in alcohol, remains, while the other matters dissolve. After drying at 120° C., the residue may be weighed, when its weight, multiplied by 0·7402, gives that of the lactic acid. Zinc paralactate dissolves readily in alcohol, so the above process is useless for the determination of paralactic acid. With inactive lactic acid it yields fairly approximate results, with careful manipulation and under favourable circumstances.

According to R. Palm (*Zeit. anal. Chem.*, xxvi. 33; abst. *Jour. Chem. Soc.*, 1887, p. 307), when treated with lead acetate and alcoholic ammonia, lactic acid is completely thrown down as a heavy granular precipitate of the formula $3PbO,2C_3H_6O_3$. To examine an animal or vegetable organ for free lactic acid, Palm extracts it with ether (previously acidulating with sulphuric acid if a lactate is under treatment), evaporates the ethereal solution to a syrup, and treats the residue with water. The filtered aqueous solution is mixed with lead acetate, and any precipitate produced is filtered off. On adding more lead acetate to the filtrate, followed by alcoholic ammonia, the lactic acid is said to be thrown down free from foreign substances. The precipitate may be washed with alcohol, in which it is quite insoluble, and the contained lactic acid estimated from the loss on ignition. Minute traces of lactic acid may be thrown down with greater certainty by shaking the filtrate from the first lead precipitate with an excess of freshly precipitated lead hydroxide. In either case the precipitate is stated to yield pure lactic acid when decomposed by sulphuretted hydrogen and extracted with ether.

W. Windisch[1] (*Chem. Centralb.*, 1887, page 826) proposes to detect small quantities of lactic acid by treating the substance with chromic acid, whereby formic acid and aldehyde are produced. The solution to be tested is diluted to about 100 c.c., 5 c.c. of concentrated sulphuric acid and a little potassium bichromate added, and the liquid distilled. The vapours are received in warm Nessler's solution, with which, in presence of aldehyde, lead salts give a yellowish-red precipitate, or with smaller quantities a yellowish opalescence. Formic, acetic, propionic, butyric, valeric, succinic, malic, citric, and tartaric acids are said not to give the reaction, but alcohol, ammonia, and sugar must be absent. To examine roots for lactic acid, they are first exhausted with ether, which is said to extract all substances of an acid nature.

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[1] Windisch's method appears to be very unsuitable for the detection of small quantities of lactic acid, since an excess of chromic acid will infallibly oxidise the formic acid and aldehyde to carbonic and acetic acids.

A method of determining lactic acid described by Chapman and Smith (*Jour. Chem. Soc.*, xx. 173) is based on the fact that, when heated with sulphuric acid and bichromate of potassium, it is decomposed thus:—$2C_3H_6O_3 + O_2 = 2C_2H_4O + 2H_2O + 2CO_2$. Hence, one-third of the carbon of the lactic acid is evolved as carbon dioxide gas. Of course, the process is only applicable in the absence of interfering substances, which are somewhat numerous. Alcohol (if not present in large amount), acetic acid, and sulphurous acid have no disturbing influence. The process is conducted by the authors as follows:—A flask, having a side-tubulure, is connected by the latter with a small bulb-apparatus filled with concentrated sulphuric acid and immersed in cold water. The bulb-apparatus is connected with bulbs containing potash-solution (specific gravity 1·27), and beyond is a small tube containing fragments of caustic potash. The neck of the flask is closed by a perforated cork, through which is passed the stem of a tapped funnel. A weighed quantity of the lactate or solution of lactic acid is placed in the flask, and 150 c.c. of a solution containing 100 grammes of bichromate of potassium and 125 of concentrated sulphuric acid to the litre, are introduced through the tap. The contents of the flask are then heated by hot water (not by a flame). Carbon dioxide gas is evolved, which bubbles through the sulphuric acid (being thus freed from aldehyde vapour), and is absorbed by the potash solution. When no more bubbles pass, air is drawn through the apparatus, and the potash apparatus is removed and weighed. Its increase in weight, corresponding to the CO_2 produced, multiplied by 2·045, gives the amount of lactic acid present.

COMMERCIAL LACTIC ACID.—The strength of lactic acid, in the absence of other acids, may be ascertained by titration with caustic alkali, with phenol-phthaleïn as the indicator. The acid should be diluted with water, and the titration conducted in the cold and as rapidly as possible, the end-reaction being the point when a pink coloration is produced which remains after stirring. 1 c.c. of normal soda neutralises 0·090 gramme of lactic acid. As stated on page 411, commercial lactic acid contains more or less lactic anhydride, which does not immediately neutralise alkali when added to the cold solution of the acid, but reacts when the sample is boiled for some time with excess of caustic alkali. This behaviour affords a means of determining the proportion of lactic anhydride in a sample of lactic acid. The acid is first treated with dilute caustic soda until exactly neutral to phenol-phthalein, and then boiled for twenty minutes in a flask furnished with a long tube with a known volume of standard caustic soda. The liquid is then titrated back with standard acid, when the deficiency of alkali represents that which has reacted with the lactic anhydride. One c.c. of normal caustic alkali corresponds to 0·081 gramme of $C_6H_{10}O_5$.

Besides water and lactic anhydride, commercial lactic acid is liable to contain the following impurities:—

Inorganic matters, left on igniting the substance. Sulphuric acid and *sulphates* will be indicated on adding barium chloride to the aqueous solution of the original substance; *chlorides* by silver nitrate; salts of *calcium* by ammonium oxalate; *zinc*, *lead*, and *iron* by diluting the liquid, nearly neutralising with ammonia, and passing sulphuretted hydrogen.

Foreign organic acids.—Of these, the presence of *oxalic* or *tartaric acid* will be indicated by the formation of a precipitate on adding lime-water to alkaline reaction, and *citric acid* by precipitation occurring on boiling the liquid so obtained. *Acetic* and *butyric acids* may be recognised by their respective odours on gently heating the liquid; or more certainly by the production of the fragrant odours of their respective ethyl ethers on heating the sample with alcohol and strong sulphuric acid. Ethyl lactate boils at a high temperature and has very little odour.

Sarcolactic acid may be detected by the formation of a blue *precipitate* on adding cupric sulphate to the aqueous solution of the substance.

Neutral organic matters may, in general, be detected by the production of a brown colour on mixing the sample with an equal measure of cold concentrated sulphuric acid. The United States Pharmacopœia (1890) requires that the tint produced by such treatment shall not be deeper than a pale straw colour. *Glycerin* may be detected by treating the sample with a slight excess of zinc oxide and a little water, evaporating to dryness at 100°, and treating the residue with ether-alcohol. On evaporation of the solution, glycerin will be left as a sweet syrupy liquid. On treating the residue left undissolved by ether-alcohol with alcohol alone, *cane-sugar* and *glucose* will be dissolved. Glucose and other impurities will also be recognised by the formation of a red or yellow precipitate on heating the neutralised acid with Fehling's solution.

Lactic acid should not be materially coloured when heated with a strong solution of caustic alkali, and should be wholly soluble in ether.

Lactic acid has recently been prepared on a large scale and employed in textile colouring as a substitute for tartaric and citric acids, over which it presents certain advantages. It is said to be a good substitute for sulphuric or acetic acid in mordanting wool with bichromate; and is stated to fix chrome better than any other acid hitherto used. As met with in commerce, the lactic acid intended for these purposes has a strength of about 43 per cent., is nearly colourless, and contains calcium sulphate and a faint trace of iron.

METALLIC LACTATES are all more or less soluble in water, but usually dissolve only sparingly in the cold. They are all insoluble in ether. The paralactates are usually more soluble than the salts of ordinary lactic acid, and are lævo-rotary.

Calcium Lactate, $Ca(C_3H_5O_3)_2$, is obtained in crystals containing 5 aq. when lactic acid is neutralised with lime or chalk, and the liquid concentrated. It crystallises in small white mammillated tufts, which under the microscope appear as delicate rhombic needles, some of which look like bundles bound in the centre. From acid solutions, a so-called *acid lactate* of calcium (a compound of calcium lactate with lactic acid) crystallises in radiating trimetric needles or fibrous masses. One part of calcium lactate dissolves in 9½ parts of cold water, and in all proportions in boiling water or alcohol (compare calcium sarcolactate, page 421). When calcium lactate is heated, it readily parts with its water of crystallisation, and at 250° to 260° is converted into a tumefied mass, containing calcium dilactate, $Ca(CO_2)_2.O(CH_3.CH)_2$, from which absolute alcohol dissolves out any unaltered lactate, leaving the dilactate as a sparingly soluble residue. The corresponding dilactic acid is unknown.

Ferrous Lactate, $Fe(C_3H_5O_3)_2$, crystallising in light yellow needles with 3 aq., soluble in 48 parts of cold or 12 parts of boiling water. The dry salt is permanent, but the solution rapidly oxidises. Ferrous lactate is not unfrequently adulterated, the substances used for the purpose being dried ferrous sulphate, milk-sugar, and starch. A sample of "lactate of iron," examined by M. Peltier, contained 25 per cent. of ferrous sulphate and 75 per cent. of milk-sugar. *Ferrous sulphate* can be readily detected by the copious precipitate produced on treating the solution of the sample with barium chloride. *Milk-sugar* may be detected by rendering the solution alkaline by soda, passing sulphuretted hydrogen to precipitate the iron, filtering, adding Fehling's solution to the filtrate, filtering rapidly in the cold from the copper sulphide, and heating the filtrate, when a yellow or red precipitate of cuprous oxide will be formed if milk-sugar be present. *Starch* may be detected in the portion of the sample insoluble in cold water, by the blue colour produced on addition of solution of iodine.

Lead Lactate, $Pb(C_3H_5O_3)_2$, is freely soluble in water, sparingly soluble in cold, but readily in hot alcohol, and slightly soluble in ether. (*Glycerate* of lead is but slightly soluble in cold water.) By adding lead acetate and alcoholic ammonia, lactic acid is completely precipitated as a compound containing $3PbO,2C_3H_6O_3$ (compare page 412).

Zinc Lactate, $Zn(C_3H_5O_3)_2$, crystallises from concentrated solutions in shining crusts, or from dilute solutions in four-sided prismatic needles, soluble in 58 parts of cold or 6 of boiling water, and insoluble in alcohol (compare zinc sarcolactate, page 421). The crystals contain 3 aqua, which is lost rapidly at 100° C., and above 210° C. the salt decomposes.

Lactic Acid in Digestion.

The statements published as to the presence of lactic acid in the gastric juice are very conflicting, some observers contending that it is a normal and constant constituent of that secretion; others wholly denying its presence in a state of health; and a third section maintaining that it is present only during a certain period of the process of digestion, being then derived from the food and not from the secretion itself. The chemistry of digestion has been recently investigated by Ewald and Boas, whose results seem to show that in the digestion of lean meat by the human stomach three distinct stages can be recognised. During the first stage, which may continue for fifty minutes after ingestion, lactic acid is the only free acid present. Ewald and Boas believe that this lactic acid is simply that which pre-existed in the meat, and is not secreted by the stomach, since when egg-albumen was substituted for meat they could not find lactic acid by any test, though hydrochloric acid was readily detected. During the second stage of digestion (that is, from sixty to ninety minutes after ingestion), lactic and hydrochloric acids occur together; while in the third and most active stage, which is at its height in about two hours, hydrochloric acid is alone met with, the lactic acid having been absorbed or destroyed in some way not understood.

he same succession of changes occurs during the digestion of
ɪd food, but in this case inactive lactic acid is also formed by
fermentation of the carbohydrates. The better the working
lition of the stomach, the sooner does the lactic acid disappear
hydrochloric acid take its place; whereas in the commoner
is of acid dyspepsia[1] lactic acid is present in undue amount,
its disappearance and its replacement by hydrochloric acid
lelayed. In such cases butyric and acetic acids occur with
lactic acid, and the hydrochloric acid is relatively small in
ntity. Cane-sugar is readily converted into glucose in the
ach, and the glucose, by the action of the fermentation-
nisms always present, is subsequently transformed into lactic

In course of time, a further transformation may supervene,
lactic acid splitting up into butyric acid, carbonic acid, water,
hydrogen. The stomach under these circumstances contains
læ, *sarcinæ*, and many other vegetable organisms in abund-
. The gas-forming organism appears to be a *bacillus*, which
been isolated and cultivated in Pasteur's fluid by M'Naught.

n some cases it is of interest to ascertain the proportions, rela-
and absolute, of the different acids present in the stomach,
h is best effected as follows:—A sample is obtained by the
f the stomach-pump, and 50 c.c. of the filtered liquid distilled
l a volume of about 35 c.c. has passed over. The residue is
ted to 50 c.c. with water and again distilled to about 10 to
.c. In the distillate, the *volatile acids* (butyric, acetic, &c.)
letermined by titration with caustic alkali, phenol-phthaleïn
g used as the indicator. The residue in the retort is shaken
or six times with large quantities of ether (500 c.c.), which
acts the lactic acid, while the aqueous liquid contains the
ochloric acid. The lactic acid is best recovered by distilling
ether to a small bulk in presence of a slight excess of caustic
, separating the remaining ether, and further evaporating the
ous solution of sodium lactate. On then acidulating with
huric acid, the lactic acid is set free, and can be extracted by
ated agitation with ether. It can be recovered by evaporat-
he ether, or alcohol and phenol-phthaleïn may be added and
liquid titrated with standard caustic soda.

he hydrochloric acid contained in the aqueous liquid separated
the first series of treatments with ether can be determined
tration with caustic alkali and litmus, but an excessive result
be obtained if acid phosphates are also present, as is some-
s the case. An equally rapid and accurate determination of

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cid dyspepsia is occasionally due to an excessive production of hydro-
ic acid.

the hydrochloric acid can be made by employing methyl-orange as an indicator. Since this reagent is neutral to acid phosphates of the formula MH_2PO_4, these salts do not interfere. One c.c. of decinormal caustic soda represents 0·00365 gramme of HCl. The phosphates may be subsequently determined in the same liquid which has been made neutral to methyl-orange, by adding a few drops of phenol-phthaleïn solution and continuing the addition of standard caustic alkali until a pink coloration is produced. The reaction being $NaH_2PO_4 + NaHO = Na_2HPO_4 + H_2O$, each c.c. of decinormal alkali employed represents 0·0081 gramme of P_2O_5.

When it is merely desired to determine the lactic acid without actually isolating it in an approximately pure state, the liquid (preferably freed from volatile acids by distillation) may be titrated with decinormal caustic soda until exactly neutral to litmus, then evaporated to dryness, and the residue heated to dull redness, without attempting to burn off the whole of the carbon. The charred mass is boiled with water, and the filtered solution titrated with decinormal acid and litmus (or methyl-orange). The amount of acid required corresponds to the sodium carbonate produced by the ignition of the lactate. Hence 1 c.c. of decinormal acid used represents 0·090 gramme of *lactic acid* in the liquid employed. The determinations of lactic acid made in this manner are somewhat above the truth, especially if acid phosphates were present in notable amount,[1] but they are sufficiently accurate for many purposes. The measure of decinormal acid required for the neutralisation of the ash, deducted from the volume of decinormal alkali required to neutralise the original liquid, corresponds to the hydrochloric acid present, which may thus be indirectly determined with approximate accuracy.

W. von Moracewski (abst. *Analyst*, 1896, p. 74) determines free hydrochloric acid in gastric juice by evaporating a known measure of the sample to 1 c.c. on the water-bath, rinsing it into a 100 c.c. flask with 25 c.c. of absolute alcohol, and filling the flask to the mark with dry ether. All the chlorides are precipitated, and the free hydrochloric acid in solution can be determined by neutralising the liquid, distilling to a small bulk, and titrating with standard silver nitrate, using neutral potassium chromate as an indicator.

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[1] According to Leo, the error from this source may be avoided by shaking the liquid to be tested with pure calcium carbonate till neutral to litmus, instead of titrating it with caustic soda. Acid phosphates do not react with calcium carbonate at the ordinary temperature, whereas hydrochloric acid and lactic acid are readily neutralised. The ash contains calcium carbonate in amount corresponding to the lactic acid previously present, and must be extracted with a known measure of standard acid, the excess of which is afterwards determined.

Where it is only required to detect the presence of hydrochloric acid in gastric juice, this may be conveniently effected by the azo-dye known as tropæolin OO (diphenylamine-yellow, Part i. page 189). A solution of the colouring matter is made in methylated spirit of 60° O.P., and drops of the liquid placed on a porcelain slab and dried at 40° C. A drop of the gastric juice or other fluid to be tested is then placed on each spot of dye, still at 40° C., when a violet coloration will be observed after the liquid has evaporated, if hydrochloric acid be present. One drop of a solution containing 0·006 per cent. of HCl is said to give a distinct reaction.

Wiesner and Singer recommend that a few drops of the filtered gastric juice should be evaporated with an equal measure of a reagent prepared by dissolving 1 part of vanillin and 2 of phloroglucol in 30 of rectified spirit. Red crystals (or in presence of peptone a red paste) will be formed if the juice contain 1 part in 10,000 of hydrochloric acid.

For the detection of free lactic acid in gastric juice, Uffelmann (*Zeit. klin. Med.*, vii. 392) prepares a reagent by mixing 10 c.c. of a 4 per cent. solution of carbolic acid with 20 c.c. of water, and adding 1 drop of solution of ferric chloride B.P. This forms a clear liquid of an amethyst colour, which is turned yellow by a solution of lactic acid containing only 1 part in 10,000. Hydrochloric acid in small quantities has but little effect, and when present in large amount simply decolorises the reagent. The test is preferably applied to the ethereal extract obtained as described on page 417, as in that case the traces of thiocyanates normally present in the stomach do not interfere.

Active Lactic Acids.

By adopting the principle previously applied by Pasteur to the preparation of dextro-rotatory and lævo-rotatory tartaric acids from inactive racemic acid, T. Purdie (*Jour. Chem. Soc.*, lxi. 754) found that ordinary inactive lactic acid could be decomposed into two oppositely active lactic acids by fractional crystallisation of the strychnine salt, that of the lævo-rotatory acid being the less soluble. The two free acids exhibit equal and opposite optical activities to those of their salts. The zinc salts of each of the optically active acids crystallise with $2H_2O$. On mixing aqueous solutions of equal quantities of the two zinc salts, and stirring the liquid, inactive zinc lactate containing $3H_2O$ separated.[1] The two optically active modifications of lactic acid present a very close resemblance to ordinary lactic acid and to each other. The anhydride and salts of dextro-lactic acid are lævo-rotatory, while the anhydride and salts of lævo-lactic acid are dextro-rotatory. On heating, both active modifications yield the same lactide, and when this takes up the elements of water, it yields ordinary inactive lactic acid. Lævo-lactic acid is stated by Lewkowitch (*Ber.*, 1883, p. 2720) to be produced by growing the mould *Penicillium glaucum* in a solution of ammonium lactate. A repetition of this experiment by Linossier (*Ber.*, xxiv. 660) gave an opposite result, the residual lactic acid yielding lævo-rotatory salts. F. Schardinger (*Vienna Academy of Sciences*) has obtained lævo-lactic acid by the fermentation of cane-sugar by a *schizomycetes* closely resembling the ordinary lactic acid bacillus in appearance, but of considerably greater fermentative power.

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[1] The existence of the physical isomerides of ordinary lactic acid affords

DEXTRO-LACTIC ACID. SARCOLACTIC ACID. PARALACTIC ACID. This acid is obtainable in the manner above described from inactive lactic acid. It occurs naturally in the juices of muscular tissue, in bile, and in the urine of persons poisoned by phosphorus.[1] It is also said to have been obtained in numerous impure fermentations (*e.g.*, of dextrin, glucose, cane-sugar, milk-

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strong support to the interesting theory of stereo-isomerism. It has been suggested with great plausibility by Le Bel and van't Hoff that the dextro- and lævo-rotatory acids are related to each other in the same manner that an object is related to its reflection in a mirror. Such isomerism requires that there shall be four dissimilar residues or radicals united to the carbon-nucleus of the molecule. This condition exists in lactic acid, in which the nucleal carbon-atom has its four bonds satisfied respectively with H, CH_3, OH, and CO_2H (see formula on page 408); and a similar asymmetric carbon-atom exists in the molecules of active amylic alcohol, active valeric acid, malic acid, asparagine, and aspartic acid. Each of these bodies can exist as a *dextro-* and as a *lævo*-rotatory modification, and the two modifications of opposite rotation can combine together in equal quantities to form an *inactive* form capable of resolution into two active varieties. It follows that compounds containing one asymmetric carbon-atom can form three physical isomerides, two active and one inactive, while in compounds the molecules of which contain more than one asymmetric carbon-atom the number of possible isomerides is correspondingly greater. It is probable that all optically active compounds contain one or more asymmetric carbon-atoms.

[1] From recent researches, T. Irisawa (*Zeit. Physiol. Chem.*, xvii. 340) concludes that lactic acid is always present in blood removed from the dead body, and in three cases out of seven it was present in urine passed by men shortly before death. Lactic acid was always found in blood freshly drawn from the veins of a dog, and its presence was also noted in pus and in blood-corpuscles. In artificially induced anæmia, the amount of lactic acid in the blood rose in proportion to the lessening of oxidation-processes.

sugar, &c.), while more recently Nencki and Sieber (*Ber.*, xxii. 695) have found it in a pure fermentation of dextrose. Frankland and Macgregor have also obtained sarcolactic acid by the interrupted bacterial fermentation of ordinary inactive calcium lactate (*Jour. Chem. Soc.*, lxiii. 1028).

Sarcolactic acid presents the closest resemblance to ordinary lactic acid. The most tangible distinctions are:—(1) The dextro-rotation of free sarcolactic acid, and the lævo-rotation of its anhydride and salts; ordinary lactic acid, both in the free state and in the form of salts, being optically inactive. (2) The solubility and amount of water of crystallisation in the calcium and zinc salts. (3) Ordinary lactic acid yields a deep blue liquid on addition of cupric sulphate, while sarcolactic acid is almost completely precipitated by that reagent.

Sarcolactic acid is conveniently prepared by dissolving extract of meat in 4 parts of water, and adding to the solution three times its volume of methylated spirit. The filtered liquid is evaporated to a syrup, which is again treated with alcohol, filtered, evaporated, acidulated with sulphuric acid, and extracted with ether. The acid thus obtained on evaporation of the ether may be purified by conversion into the zinc salt.

On heating, sarcolactic acid yields a lævo-rotatory anhydride, the solution of which is lævo-rotatory; but this active anhydride and the lactide formed on further heating are hydrolysed by water to a solution of ordinary inactive lactic acid.

Calcium sarcolactate, $Ca(C_3H_5O_3)_2 + 4H_2O$, is soluble in about 12 parts of cold water. The solution is lævo-rotatory, the value of $[\alpha]_D$ for a solution containing 5·35 per cent. of the anhydrous salt being stated by Wislicenus at $-5·48°$.

Zinc sarcolactate, $Zn(C_3H_5O_3)_2 + 2H_2O$, crystallises in slender needles which lose their water slowly at 100°, and give off empyreumatic vapours below 150°. The salt is soluble in 16 parts of cold water, but only sparingly soluble in cold alcohol (distinction from ethylene-lactic acid). The aqueous solution of zinc sarcolactate is lævo-rotatory, the following being the values for $[\alpha]_D$ found by Wislicenus for solutions of various strengths:—

Grammes of anhydrous salt per 100 c.c., . .	4·58	4·75	5·36	6·51	9·60	13·98
Specific rotation $[\alpha]_D$, .	8·73°	8·43°	8·49°	7·83°	7·29°	7·30°

Ethylene Lactic Acid. Hydracrylic Acid.

β-Hydroxypropionic Acid.

$C_3H_6O_3$; *i.e.*, $OH.CH_2.CH_2.COOH$

This compound is distinguished from the other modifications of lactic acid by the fact that it yields no trace of lactide when heated, being resolved, almost without residue, into water and acrylic acid, $C_3H_5.COOH$. The same decomposition occurs on heating it with sulphuric acid diluted with an equal weight of water. On the other hand, when acrylic acid is heated to 100° with excess of caustic soda, hydracrylic acid is reproduced. Hydracrylic acid has only been obtained by synthetical means,[1] its formation from β-iodopropionic acid by boiling with water or heating with moist silver oxide being the most available reaction.

On oxidation with nitric acid, hydracrylic acid yields carbon dioxide and oxalic acid. With chromic acid mixture, the former is the sole product of the reaction.

The sodium and calcium salts of hydracrylic acid melt without change at about 140° to 145°, but at a higher temperature they lose water and are converted into acrylates. *Zinc hydracrylate* crystallises in large shining prisms, containing 4 aqua, soluble in an equal weight of cold water. The salt is also soluble in alcohol, which precipitates zinc lactate and sarcolactate from their aqueous solutions.

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[1] Wislicenus obtained from flesh, together with sarcolactic acid, an acid which he supposed to be ethylene-lactic acid, but it has been shown by Siegfried (*Ber.*, xxii. 2713) that this was really acetyl-lactic acid.

CYANOGEN AND ITS DERIVATIVES.

THE members of the cyanogen group are among the most remarkable and best known compounds in the domain of organic chemistry, which has been aptly termed "The Chemistry of Compound Radicals."

Cyanogen itself is not only obtainable in a free state, but enters into direct combination with the metals and unites with hydrogen to form a compound of acid properties. Cyanogen unites with the halogens, and also forms an oxy-acid and thio-acid, each of which yields a well-defined series of salts. Cyanogen presents greater analogies to iodine than to any other element,[1] but has special chemical peculiarities which are without parallel in the case of any other radical, either simple or compound.

The metallic cyanides are an interesting and important class of salts, and some of them receive an extensive industrial application. The cyanides of many of the heavy metals combine with the cyanides of the light metals to form compounds which in many instances are of an exceedingly stable character. Hence the properties of these double cyanides are often much modified, and many of them do not respond to the tests for simple cyanides or for hydrocyanic acid (see page 456).

All bodies containing cyanogen, in whatever form of combination it may be, yield the whole of their nitrogen in the form of ammonia when they are strongly ignited with excess of soda-lime. The whole of the nitrogen is also converted into ammonia when a compound of cyanogen is strongly heated with concentrated sulphuric acid, as in Kjeldahl's process.

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[1] The following table shows the analogy between cyanogen and chlorine :—

Chlorine, ClCl.	Cyanogen, CyCy.
Hydrochloric Acid, HCl.	Hydrocyanic Acid, HCy.
Potassium Chloride, KCl.	Potassium Cyanide, KCy.
Potassium Hypochlorite, KClO.	Potassium Oxycyanide (cyanate), KCyO.

CYANOGEN GAS. DICYANOGEN.

Cyanogen contains carbon and nitrogen united in atomic proportions. Hence its formula is,—$C\equiv N$, or when uncombined, $N\equiv C.C\equiv N$. The group CN is often expressed by the symbol Cy.

Cyanogen gas is one of the products of the dry distillation of ammonium oxalate, and may be obtained comparatively pure if phosphoric anhydride be added to assist the dehydration:—$(NH_4)_2C_2O_4 = 4H_2O + C_2N_2$. Cyanogen is produced in many other organic reactions. It occurs in small proportion in blast-furnace gases, and is formed when a mixture of coal-gas and ammonia is burnt in a bunsen burner.

Cyanogen gas is usually prepared by heating mercuric cyanide to dull redness:—$Hg(CN)_2 = Hg + C_2N_2$.[1] A mixture of one part of potassium cyanide with three parts of mercuric chloride also yields cyanogen gas when heated. Two parts dry potassium ferrocyanide may be substituted for the potassium cyanide, but in this case the gas evolved contains free nitrogen.

Cyanogen is a heavy colourless gas, smelling at once of bitter-almonds and of chlorine, and burning when kindled with a characteristic peach-coloured flame. Cyanogen gas liquefies under the influence of cold or pressure (5 atmospheres at 20° C.), forming a colourless liquid boiling at $-20·7°$ C. When further cooled, cyanogen solidifies to a crystalline mass melting at $-34·4°$.

When exploded with oxygen in the eudiometer, cyanogen yields its own measure of nitrogen and twice its measure of carbon dioxide:—$C_2N_2 + 2O_2 = N_2 + 2CO_2$. As the carbon dioxide formed occupies the same volume as the oxygen which has entered into combination, the volume of gas is unaltered by the explosion.

At the ordinary temperature cyanogen gas is soluble in four volumes of water or in about twenty-three volumes of alcohol. On keeping, the aqueous solution becomes turbid, and deposits a brown substance called azulmic acid, while, according to Wöhler, the liquid contains ammonium oxalate, together with smaller quantities of urea and ammonium carbonate and cyanide.

Cyanogen is absorbed with moderate facility by a solution of caustic alkali, with formation of a cyanide and cyanate:—

$$C_2N_2 + 2NaHO = NaCN + NaCNO + H_2O.$$

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[1] PARACYANOGEN, C_nN_n, is a brown amorphous substance left when cyanogen is prepared by heating mercuric or argentic cyanide. It is also formed by the spontaneous decomposition of hydrocyanic acid. When heated in a current of carbon dioxide or nitrogen, paracyanogen gradually volatilises as cyanogen gas, which may be absorbed by a solution of potash.

Glacial acetic acid, or even acetic acid of 80 per cent., is a verful absorbent of cyanogen gas, dissolving eighty times its ume.

Cyanogen gas is absorbed by a 3 per cent. solution of hydrogen oxide, oxygen being evolved after a few minutes, and on lition of a drop of solution of caustic potash oxamide separates needles. According to B. Radziszewski (*Ber.*, xviii. 355), reaction is unaccompanied by the formation of bye-products, is a general one for nitriles, occurring very readily in alkaline tion at about 40° C. The following general equation expresses change :—$R.CN + 2H_2O_2 = R.CO(NH_2) + H_2O + O_2$.

Aniline also readily absorbs cyanogen with formation of ananiline, and has been recommended by G. Jacquemin the determination of cyanogen in mixed gases. This suggestion but little practical utility, since M. Loeb has shown (*Jour. em. Soc.*, liii. 812) that the simultaneous presence of either bon monoxide or carbon dioxide would vitiate the result, by sing partial decomposition of the cyananiline and evolution of rocyanic acid.

For the determination of the traces of free cyanogen sometimes sent in crude coal-gas and the waste gases from blast-furnaces, Leybold (abst. *Jour. Chem. Soc.*, 1891, p. 367) uses the owing method :—In each of a series of three Woulffe's bottles laced 20 c.c. of a strong solution of caustic soda (1 : 3), to which dded a quantity of suspended ferrous hydroxide, prepared by ling 20 c.c. of the soda solution to 30 c.c. of a 10 per cent. tion of ferrous sulphate ; the first bottle receiving 25 c.c., the ond 15 c.c., and the third 10 c.c. of the mixture. The gas (100 es) is passed through the apparatus during sixty to ninety utes, when the contents of the three bottles are transferred flask and boiled for fifteen minutes. The solution is allowed cool and settle, when the supernatant fluid is decanted into a c.c. flask, and the ferrous hydroxide well washed. The filtrate hen made up to 500 c.c., and an aliquot part (100 or 200 c.c.) dulated with hydrochloric acid, excess of ferric chloride added, the precipitated prussian blue collected and washed with ll quantities of water till free from chlorides. The filter with precipitate is then treated with dilute soda, the liquid filtered, the filtrate evaporated to dryness with excess of dilute sulphuric d. The residue is ignited and the iron in it determined. 56 parts Fe or 80 of Fe_2O_3 represent 156 parts of cyanogen.

H. Drehschmidt has described a modified process, in which alkaline contents of the Woulffe's bottles or absorption-cylinders boiled with mercuric oxide, the resultant mercuric cyanide decomposed by zinc, and the liquid titrated by silver solution (*Chem. Centralb.*, 1892, i. 1006; abst. *Jour. Chem. Soc.*, 1893, ii. 50).

Halogen-Compounds of Cyanogen.

Cyanogen unites with the halogens to form compounds in which the cyanogen is electropositive.

GASEOUS CYANOGEN CHLORIDE, CyCl, is obtained by the action of chlorine gas on hydrocyanic acid or on mercuric cyanide. It is an extremely pungent, poisonous gas, which powerfully affects the eyes. Under a pressure of four atmospheres it condenses to a colourless liquid, which at −18° C. solidifies to long transparent prisms.

SOLID CYANOGEN CHLORIDE or CYANURIC CHLORIDE, Cy_3Cl_3, forms monoclinic crystals which melt at 140° and boil at 190°. It has an acrid taste, and is a powerful poison. It has a pungent odour, and acts on the eyes. When dilute, the odour of cyanuric chloride is compared to that of the excrement of mice. Cyanuric chloride is slightly soluble in water, and is decomposed by boiling water with formation of hydrochloric and cyanuric acids. $Cy_3Cl_3 + 3H_2O = 3HCl + H_3Cy_3O_3$.

CYANOGEN BROMIDE, CyBr, is obtained by adding bromine to a cooled solution of potassium cyanide, when a mixture of potassium bromide and cyanogen bromide crystallises out. The crystals, when heated to about 60°–65°, give a sublimate of the latter compound in delicate prisms which rapidly change to cubes. Cyanogen bromide has a pungent smell, acts powerfully on the eyes, and is very poisonous. It has recently been prepared on a large scale for use in the extraction of gold by the cyanide process (see page 460).

CYANOGEN IODIDE, CyI, is formed by the action of iodine on metallic cyanides. It is produced in the process of Fordos and Gelis (page 435) for the determination of cyanogen, and is said to be a common impurity in commercial iodine.[1] Cyanogen iodide

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[1] For the detection of cyanogen iodide in iodine, C. Meineke (abst. *Jour. Chem. Soc.*, 1893, ii. 246) acidifies the solution with a drop of dilute hydrochloric acid, and adds sodium thiosulphate in such amount as to leave only a slight yellow coloration. If ferrous sulphate and an alkali be now added, a precipitate of prussian blue will be formed after again acidulating the liquid.

Meineke states that the cyanogen in commercial iodine may be determined with moderate accuracy by ascertaining the difference in the volume of a standard thiosulphate solution required in acid and in neutral solutions of the sample. If the iodine be dissolved in a solution of potassium iodide and rms white needles, soluble in alcohol and ether but only spar-gly soluble in water. It is poisonous, has an odour like that cyanogen bromide, and volatilises at ordinary temperatures ough it has a high boiling point.

When iodine is added to a solution of hydrocyanic acid, a rtain amount of cyanogen iodide is formed ($HCy+I_2=$ $I+CyI$), but beyond a certain point the iodine is no longer ted upon. The amount of iodine capable of being converted to cyanogen iodide, the amount of hydrocyanic acid remaining nstant, increases both with the temperature and with dilution. yanogen iodide is completely decomposed by hydriodic acid, lphurous acid, hydrogen sulphide, stannous chloride, and other ducing agents, but is as stable as iodic acid towards oxidising ents.

Cyanogen iodide may be determined volumetrically by either ydriodic or sulphurous acid. According to E. von Meyer, nall quantities of hydrocyanic acid prevent the reduction of iodic id by formic acid, but do not influence the reduction of iodic id by hydriodic acid.

IMPLE CYANIDES.

As stated on page 423, the metallic cyanides exhibit a great ndency to unite with each other to form highly stable combinaons, which analytically are quite distinct from their constituents. he more important of these double cyanides are considered in parate sections.

Some of the metallic cyanides receive extensive industrial plications, and much ingenuity has been expended on their onomical production. An outline of the more important ocesses of manufacturing cyanides is given under "potassium anide," which is the type of the simple metallic cyanides.

On ignition *per se* the cyanides of the noble metals are decomsed with liberation of the metal, cyanogen gas, and formation

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ydrochloric acid added, the following reaction takes place on adding iosulphate :—

$$2CyI+4Na_2S_2O_3+2HCl=2Na_2S_4O_6+2NaCl+2NaI+2HCy.$$

neutral solutions, however, 3CyI are said to react with $5Na_2S_2O_3$ with rmation of one molecule of sodium sulphate. Meineke suggests that this mpound is a product of a secondary reaction between the cyanide of alkalietal and the tetrathionate, but the reaction by which it is formed is not vious. He further proposes to deduce the cyanogen iodide from the amount sulphate formed.

of more or less paracyanogen (see page 424). The cyanides of iron are decomposed into iron carbide and nitrogen gas. The cyanides of the alkali-metals are not affected by simple ignition in the absence of air, but in the presence of oxygen they are converted into the corresponding cyanates, and in presence of vapour of water yield more or less ammonia and leave an alkaline carbonate.

Heated with potassium nitrate or chlorate, the metallic cyanides detonate strongly.

The cyanides of the metals of the alkalies and alkaline-earths are soluble in water, the former readily, the latter with difficulty. They are decomposed by atmospheric carbonic acid with liberation of hydrocyanic acid.

Cyanogen has a remarkable affinity for the noble metals. Thus metallic silver and gold dissolve in potassium cyanide in presence of oxygen. Mercuric cyanide is extremely stable, the mercury not being precipitated by caustic alkalies, nor the cyanogen by silver nitrate; but on treatment with palladious nitrate, a yellow precipitate of palladious cyanide is thrown down.

The simple cyanides of the heavy metals are insoluble in water, with the exception of mercuric and thallium cyanides (see p. 455), but their combinations with the cyanides of the alkali-metals are mostly soluble.

The *cyanides of organic radicals* are of great theoretical interest, but have no commercial importance.

DETECTION OF CYANIDES.

A solution of potassium cyanide gives the following reactions:—

1. On warming with dilute hydrochloric acid, poisonous hydrocyanic acid is evolved, having an odour of bitter-almonds.

2. Silver nitrate, added in excess, throws down silver cyanide, AgCy, as a white, curdy precipitate, exactly resembling silver chloride in appearance and general properties. The washed precipitate differs from silver chloride in the following respects:—

a. By evolving hydrocyanic acid when boiled with hydrochloric acid.

b. By dissolving in concentrated nitric acid (in which silver chloride is insoluble), yielding a solution in which silver may be detected by adding hydrochloric acid. This reaction serves to separate chloride of silver from the cyanide. If the solution of silver cyanide in hot nitric acid of 1·2 sp. gr. be filtered from the insoluble silver chloride and allowed to cool without agitation, the silver cyanide is deposited in a semi-gelatinous form. On gentle agitation it collects suddenly into opaque masses, which under the microscope appear as groups of needles.

y suffering complete decomposition with quantitative
on of ammonia, when strongly heated with fuming
ic acid and a globule of metallic mercury.

y evolving cyanogen gas on ignition, leaving metallic
mixed with paracyanogen). Silver chloride fuses without
osition.

y responding to reactions 3 and 4.

y being deposited in needles after being very gently heated
mmonia, whereas silver chloride, when similarly treated,
ctahedra.

n adding caustic soda and ferrous sulphate, a ferrocyanide
ed :—$6KCy + FeSO_4 = K_2SO_4 + K_4FeCy_6$. On acidulating
uid with hydrochloric acid and adding ferric chloride, a
a blue precipitate (or bluish-green coloration) of ferric
anide is produced. If the ferrous sulphate contain any
salt, the addition of ferric chloride is superfluous. In
for traces of hydrocyanic acid, the employment of excess
iron salts should be carefully avoided, or the yellow colour
sk the green. Properly performed, the test is very delicate
racteristic, 1 part of hydrocyanic acid in 50,000 of water
ndicated. It is not applicable in presence of ferrocyanides
cyanides.

n evaporation to dryness at a steam-heat, after addition of
ammonium sulphide,[1] a thiocyanate (sulphocyanide) is
. On treating the residue with water, filtering if necessary,
ting with hydrochloric acid and adding ferric chloride, a
ed colour is produced, due to the formation of ferric thio-
. The colour is distinguished from that due to an acetate
ng unaffected by dilute hydrochloric acid, and from that
ed by a meconate by being readily destroyed on addition of
ic chloride. If the use of excess of iron solution has been
l, on agitation of the liquid with ether the sulphocyanide
es and colours the ethereal layer red. This test can be
l to silver cyanide. Free hydrocyanic acid or a simple
e can be detected in presence of a ferrocyanide, ferricyanide
cyanate by mixing the suspected liquid with tartaric acid,
g it, and allowing the vapours to act on a drop of yellow
ium sulphide contained in a porcelain dish or watch-glass,
d over the vessel containing the sample to be tested.
ome time the cover is removed, the drop of liquid evapo-
o dryness, and the residue treated as above described. This

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en free hydrocyanic acid is to be tested for, it is advantageous to add
f a weak solution of caustic alkali before treating the solution with
um sulphide, an excess of which should be carefully avoided.

elegant and delicate test, which is due to Liebig, is said to indicate the presence of 1 part of hydrocyanic acid in 4,000,000 of water.[1]

Free hydrocyanic acid responds readily to all the above tests.

Mercuric cyanide reacts peculiarly, and must be examined in a special manner (pages 448, 455).

5. A useful test for free hydrocyanic acid is the blue coloration it produces with gum guaiacum in presence of compounds of copper. The test is commonly applied by moistening a slip of filter-paper with a very dilute solution of copper sulphate, then with a tincture of guaiacum, and drying it. If the paper so prepared be immersed in a liquid containing a trace of hydrocyanic acid it acquires a bright blue colour. Hilger and Tamba (abst. *Jour. Chem. Soc.*, 1891, page 1555) consider guaiacum test-paper untrustworthy, and recommend that the suspected liquid should be treated in a porcelain dish with a drop of tincture of guaiacum, followed by a drop of solution of copper sulphate.

DETERMINATION OF CYANIDES.

The behaviour of cyanogen compounds with indicators of neutrality presents some curious anomalies, which must be borne in mind when attempting to determine cyanides by alkalimetric methods. The following is a statement of the chief facts observed with respect to potassium cyanide and its associates in cyanide liquors of gold-extraction works :—

	Percentage of Total Potassium indicated by titration with Standard Hydrochloric Acid.		
	Litmus.	Phenol-phthalein.	Methyl-orange.
Potassium cyanide, . . .	100	100	100
Potassium sulphocyanide, .	0	0	0
Potassium ferrocyanide, .	0	0	0
Potassium ferricyanide, . .	0	0	0
Potassium zinc cyanide, . .	...	7·9[2]	200[2]
Potassium silver cyanide, .	0[3]	0[4]	—
Potassium zinc oxide, . .	100	100	100
Potassium carbonate, . .	100 (hot)	50	100
Potassium bicarbonate, . .	100 (hot)	0	100
Potassium hydroxide, . .	100	100	100

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[1] Link and Mæckel (*Zeitsch. anal. Chem.*, 1878, p. 455).

[2] This statement is made on the authority of W. Bettel (*Chem. News*, lxxii. 287). In the case of potassium zinc cyanide the point of neutrality to methyl-orange corresponds to the equation :—

$$K_2ZnCy_4 + 4HCl = ZnCl_2 + 2KCl + 4HCy.$$

[3] According to L. Siebold.

[4] According to J. E. Clennell.

Various methods of determining cyanides have been devised, but the best in most cases are those founded on the formation of argentic cyanide. The process may be conducted gravimetrically as in (1), or by one of the volumetric methods subsequently described.

1. In free hydrocyanic acid and the cyanides of the light metals, the determination of the cyanogen may be effected by treating the solid substance or liquid to be tested with an excess of a solution of silver nitrate, adding water, and then rendering the liquid moderately acid with nitric acid. The precipitate of silver cyanide is allowed to settle without applying heat, when the liquid is filtered, the precipitate washed, dried at 100°, and weighed as AgCy; or the precipitate may be ignited for a considerable time in an open porcelain crucible, and the residual metallic silver weighed.[1] 108 parts of Ag or 134 of AgCy represent 26 parts of Cy or 27 of HCy.

In presence of sulphides, these must first be removed by agitating the liquid with lead carbonate (white-lead), and adding the silver nitrate to the filtered liquid.

In presence of chloride, the silver precipitate is dried at 100° C. and weighed, then just fused, reduced by treatment with zinc and dilute sulphuric acid, filtered, and chlorine determined in the filtrate by nitrate of silver. The proportion of silver chloride corresponding thereto is deducted from the observed weight of silver precipitate, the difference being argentic cyanide. Small quantities of cyanide in a mixture with chloride are better determined by igniting the silver precipitate with soda-lime or heating it with strong sulphuric acid, and determining the resultant ammonia.

The double cyanides of potassium with nickel, copper, and zinc can be analysed gravimetrically by the above method, provided that the silver precipitate be digested for some time with silver nitrate and dilute nitric acid, the action being assisted by a gentle heat.

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[1] *Bitter-almond water* and *Cherry-laurel water* contain ammonium cyanide and benzaldehyde cyanhydrin in addition to free hydrocyanic acid. To apply the process in the text to the determination of the total cyanogen in such liquids, 50 c.c. of the sample should be treated in a stoppered bottle with excess of silver nitrate. Ammonia is then added until the liquid is strongly alkaline, when it is *at once* acidified with dilute nitric acid, carefully avoiding a large excess. The process must be performed in the cold, and with the greatest possible rapidity. Water is then added (200 c.c.), the solution agitated till the precipitate aggregates, when it is filtered off, dried, ignited, and weighed.

2. Another convenient and fairly accurate method of assaying hydrocyanic acid and preparations containing it is to make a milk by grinding magnesia with water, and add sufficient of the mixture to a known measure of the liquid to be tested to render it opaque after agitation. Two drops of a saturated aqueous solution of neutral potassium chromate are then added, and the liquid titrated with decinormal solution of silver nitrate, until a pale red tint is observed which does not disappear on agitation. This reaction corresponds to the formation of red silver chromate, which does not occur until the whole of the cyanide present has been converted into silver cyanide. One c.c. of decinormal silver solution (17·0 grammes of $AgNO_3$ per litre) corresponds to 0·0027 gramme of HCy.[1]

The foregoing process is practically identical with that commonly employed for the determination of chlorides. Hence it follows that chlorides must be absent, or must be separately determined and allowed for. A combination of the foregoing process with that next to be described would allow of a determination of chlorides in presence of cyanides.

3. A very convenient and accurate method of using silver nitrate for the determination of cyanogen in hydrocyanic acid and simple cyanides is that of Liebig. Some of the errors to which the process is liable have been pointed out by L. Siebold (*Pharm. Jour.*, [3], ix. 191), who has also extended the field to which the process is applicable, and has shown that it may be employed for the determination of alkaline cyanides in presence of free hydrocyanic acid. Liebig's process is based on the fact that silver cyanide forms a soluble double salt with potassium cyanide, of the composition AgCy,KCy. Hence the first effect of the addition of silver nitrate to a solution of a cyanide of alkali-metal is to cause the reaction :—$2KCy + AgNO_3 = AgCy,KCy + KNO_3$. Until the silver solution is added in excess of the quantity required for this reaction, the liquid remains perfectly clear, but a single additional drop causes a permanent turbidity of silver cyanide, or, in presence of chlorides, of silver chloride. The process is extremely simple, the liquid to be tested being merely placed in a flask or beaker resting on a black surface, and decinormal silver nitrate (17·0 grammes of $AgNO_3$ per litre) then run in from a burette, with continual agitation, until a slight permanent turbidity

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[1] This form of the silver process is very suitable for the assay of *bitter-almond water* and *cherry-laurel water*, as recommended by H. C. Vielhaber (abst. *Jour. Chem. Soc.*, 1879, page 280). He points out that the magnesia neutralises any free acid, and thus potassium bichromate may be substituted for the neutral salt.

ults. Each c.c. of decinormal silver solution is equivalent to 52 gramme of Cy, ·0054 of HCy, or ·01302 of KCy.

The foregoing process only indicates the metallic cyanide sent, free hydrocyanic acid giving an immediate precipitate h the silver solution. Hence, in assaying *free hydrocyanic acid*, liquid must first be treated with a full equivalent of caustic ali; but a very large excess should be avoided. The addition the proper amount cannot be ascertained by litmus, cyanides of alkali-metals having a powerfully alkaline reaction. The uid at the termination of the titration should be distinctly aline to litmus; if otherwise, more caustic alkali must be added hich will cause the disappearance of the turbidity), and the ation continued till a permanent turbidity results. To com- nsate in accurate experiments for the slight error produced by arge excess of alkali, Siebold suggests that the liquid should titrated back with normal acid, until a slightly increased bidity results. For each 1 c.c. so used, ·01 c.c. should be lucted from the volume of decinormal silver solution previously ployed.

In a liquid containing alkaline cyanide as well as free hydro- anic acid, the amounts of each of these may be ascertained by rating first without addition of caustic alkali, and then continu- the process after adding it. The volume of silver solution st used represents the metallic cyanide, the second quantity free hydrocyanic acid.

In titrating *medicinal hydrocyanic acid*, 10 c.c. of normal stic alkali (*liquor potassæ* and *liq. sodæ*, B.P., are approximately rmal) should be placed in a conical flask or beaker, diluted to 0 c.c. with water, and 10 c.c. of the sample to be tested added.[1]

Bitter-almond water 50 c.c. should be employed. It becomes lky on dilution from precipitation of hydrobenzamide, but this onvenience may be avoided by adding alcohol. A similar antity of the alcoholic solution of bitter-almond oil may be used.

Liebig's process has the advantage over method 2 that the esence of chlorides, far from interfering, is to be preferred. lphides, if present, must be previously removed by agitating e liquid with a little lead carbonate and filtering. Formates d cyanates are without influence on the results.

Bitter-almond water and old solutions of potassium cyanide are ble to give too high results by Liebig's method, owing to the esence of ammonia, which has a solvent action on the silver cyanide. Hence the end-reaction does not occur as soon as it would in the absence of ammonia. But this source of error may be wholly avoided by adding a few drops of potassium iodide to the solution. In presence of this salt the end of the reaction is indicated by a turbidity due to argentic iodide, a body which is not soluble in dilute ammoniacal liquids. The use of iodide allows ammonia to be used instead of fixed alkali for neutralising free hydrocyanic acid.

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[1] A washing bottle should be interposed between the mouth and the ette, to avoid the danger of inhaling the vapour or the fatal consequence ucking some of the liquid into the mouth.

L. Siebold has shown that chlorides when present may be conveniently determined in the same liquid in which the cyanide has been estimated by neutralising the excess of free alkali (which should not be ammonia) by the cautious addition of dilute nitric acid,[1] adding a few drops of a solution of neutral potassium chromate, and continuing the addition of the silver solution until the red tint due to the formation of silver chromate remains permanent (compare process 2). If cyanide only be present, the volume of silver solution now required will be exactly equal to that previously employed to obtain a permanent turbidity, whereas any excess over this amount represents the silver solution corresponding to the chlorides present.[2]

Liebig's process may also be applied to the partial assay of

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[1] Or a solution of magnesium sulphate may be added till the liquid is only faintly alkaline to litmus.

[2] L. Siebold has pointed out that the double cyanides of silver with the alkali-metals are very permanent bodies, while their solutions are perfectly neutral to litmus. He has based on these facts, combined with Liebig's process, an ingenious method of alkalimetry, which is directly applicable to the assay of alkaline carbonates. To the solution of a weighed quantity of the sample of carbonate, excess of hydrocyanic acid is added, and the liquid is at once titrated with standard silver nitrate. As the production of permanent turbidity is only deferred in proportion to the amount of alkaline cyanide present (the excess of the hydrocyanic acid having no effect), and as carbonates are completely decomposed by hydrocyanic acid in presence of silver nitrate, the volume of the latter solution added at once indicates the amount of alkali present, the reaction being as follows :—

$$Na_2CO_3 + 2HCy + AgNO_3 = NaNO_3 + NaCy, AgCy + H_2O + CO_2.$$

Hence, each c.c. of decinormal $AgNO_3$ employed represents ·0062 of Na_2O, or ·0106 of Na_2CO_3 in the sample taken. By boiling off the excess of free hydrocyanic acid and titrating again with silver nitrate, using neutral potassium chromate as an indicator, the proportion of chloride can be determined in the same quantity. Siebold's test analyses are remarkably good, and he claims that the alkali and chlorine in a sample of soda-ash can both be determined in ten or fifteen minutes by the use of a single burette and standard solution. Strong hydrocyanic acid for use in the above process can readily be kept permanent by addition of glycerin (*Pharm. Jour.*, [3], ix. 191).

ectro-plating liquids, which commonly consist of a solution of e double cyanide of silver and potassium. A considerable extra nount of cyanide of potassium is often present. This *only* will indicated by titrating with silver nitrate until a permanent rbidity results; 170 of $AgNO_3$ representing 130·2 of free KCy. he potassium cyanide existing in combination with silver cyanide best determined by Hannay's process, or by estimating the lver itself (p. 441).

Any carbonate existing in alkaline cyanides or plating solutions ay be determined by precipitating the liquid with calcium loride, and collecting and weighing the resultant calcium carnate.

4. J. B. Hannay (*Jour. Chem. Soc.*, xxxv. 245) has described process for the volumetric determination of cyanides based on the omalous characters of mercuric cyanide. This compound is not composed by alkalies, and is at once produced on adding a ercuric solution to that of an alkaline cyanide. In practice, e process is conducted by adding a moderate excess of ammonia the solution to be tested, placing the vessel on a black surface, d running in a decinormal solution of mercuric chloride (conining 13·55 grammes of $HgCl_2$ per litre) with continual itation until a permanent bluish-white opalescence is produced. e reaction is as follows:—$2KCy+HgCl_2=HgCy_2+2KCl$. ence, each c.c. of mercurial solution (of the above strength) nployed represents ·00651 gramme of KCy, or ·0026 of Cy, in e liquid examined. Very large proportions of ammonia interre somewhat, but less than fifteen equivalents are stated to be ithout important effect. Alkaline sulphates, nitrates and lorides do not interfere, nor do caustic or carbonated alkalies. yanates and thiocyanates are also without influence on the sults, and the same is true of silver salts, so that the total anogen existing in electro-plating liquids can be directly deterined by the process. Siebold expresses a high opinion of the curacy of Hannay's method, but the author's own experiments ow that the process, though very convenient for certain purposes, ves results only approximately correct.

5. An accurate and simple process of determining cyanogen isting in the form of a cyanide of a light metal has been devised Fordos and Gelis. It is based on the formation of cyanogen dide. The solution is simply titrated with a decinormal solution iodine, using starch solution as an indicator, until the producn of a permanent blue tint indicates the end of the reaction:— $Cy+I_2=KI+CyI$. Hence 1 c.c. of decinormal iodine reprents 0·0135 gramme of cyanogen, or 0·03255 gramme of

potassium cyanide. Sulphides must be absent, and any alkalies or monocarbonates must be neutralised by addition of excess of carbonic acid water (ordinary seltzer or soda water). Further information respecting the practical applications of the method will be found below.

Assay of Cyanide Liquors in Gold Works.

In the extraction of gold by the cyanide process it is necessary for advantageous working to ascertain the strength of the dilute solutions of potassium cyanide employed. After repeated use these are greatly reduced in strength, becoming contaminated with various impurities (*e.g.*, zinc, ferrocyanides, &c.), some of which quite invalidate the ordinary methods of assay.[1] The presence of zinc is especially objectionable, since the potassium zinc cyanide cannot be readily determined and prevents a correct titration of the potassium cyanide either by the silver or the iodine process.

In the practical assay of such dilute cyanide liquors no method

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[1] W. Bettel (*Chem. News*, lxxii. 299) has published the following analyses of spent liquors resulting in practice from the cyanide process of gold extraction. A represents a cyanide solution which had been in contact with clean pyrites from Robinson concentrates for twenty-eight months, with a limited supply of air. B was a solution from the treatment of dry crushed Robinson G. M. Company's ore without addition of neutralising agents, after passing through the zinc boxes. C was similar to B, but lime had been added, and it had not passed through the zinc box. D was like B, but with lime in small quantity, and was sampled after passing the zinc box. The figures are percentages.

	A	B	C	D
Potassium cyanide, ...	·005	·085	·24	·23
Potassium sulphocyanide, . .	·14	·004	·008	·004
Potassium ferrocyanide, . . .	·77	·074	trace	[illegible]
Potassium ferricyanide, . . .	...	...	·033	...
Potassium zinc cyanide, . . .	...	·25	...	·134
Potassium zinc oxide (K_2ZnO_2), .	...	·15	...	...
Potassium bicarbonate, . . .	·33	·566	...	·547
Ammonia, ...	·21	·808	·003	·006
Calcium hydroxide, ...	...	...	·067	...

A contained sulphates and formates, but the amounts were not determined. The carbonate in this solution was calculated to K_2CO_3. There was no sulphide present. C originally contained 0·30 per cent. of potassium cyanide and D 0·45 per cent. Free hydrocyanic acid to the amount of 0·04 per cent. is said to have been present in D. Sulphates were absent.

is admissible which does not give a perfectly definite end-reaction and which is not easily and rapidly executed. The problem has been recently investigated by J. E. Clennell (Chief Chemist to the Rand Central Ore Reduction Company, Johannesburg) and W. Bettel, of whose results the following is an epitome (*Chem. News*, lxxi. 274; lxxii. 227, 286, 298).

Solutions are frequently met with which are turbid from the presence of suspended matters. Clarification can be readily effected by agitation with lime, but such treatment is inadmissible since it causes decomposition of the zinc cyanide, and thus raises the apparent strength of the solution. On the other hand, the decomposition it effects is always incomplete, so that it cannot be employed to eliminate the zinc.

In the absence of zinc, turbid cyanide solutions may in many cases be accurately titrated, without filtering, by the iodine method. But it is essential that the solution contain no alkaline hydroxide or monocarbonate. To eliminate these, Clennell proceeds as follows:—Silver nitrate is first added to a measured volume of the cyanide solution to be tested until a permanent turbidity, or if the solution was originally turbid a distinct increase of turbidity, is observed. Addition of a few drops in excess is immaterial. A drop of phenol-phthalein solution is now added, and the liquid titrated with decinormal hydrochloric acid until the pink colour disappears. Another (equal) measured portion of the cyanide solution is then taken, and decinormal hydrochloric acid added drop by drop, with agitation, in volume a trifle less than that previously found necessary to render the other portion neutral to phenol-phthalein. This neutralised solution is then ready for titration with iodine.[1]

In the case of cyanide liquors containing zinc in notable amount, Clennell recommends the following process for the determination of the total cyanide:—A measured volume of the solution is made strongly alkaline with caustic soda, and sulphuretted hydrogen passed until it ceases to give a precipitate, or, preferably, a solution of sodium sulphide is added in slight excess.[1] The liquid is then made up to a definite volume, agitated, and allowed to stand till the zinc sulphide has settled, a little lime being added, if necessary, to assist the clarification. The solution is then filtered, and the filtrate agitated with litharge, added in small quantities at a time, with constant agitation, until a drop of the liquid no longer gives the slightest coloration with lead acetate. The liquid is then passed through a dry filter, and a known measure of the filtrate titrated with silver nitrate.

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[1] This process depends on the following facts:—

1. In a solution containing cyanide, hydroxide, and monocarbonate of alkali-metal, addition of a dilute hydrochloric acid converts the hydroxide into chloride and the monocarbonate into bicarbonate, before any cyanide is decomposed.

2. Bicarbonates of the alkali-metals are without action on iodine.

3. When sufficient silver nitrate has been added to a solution to convert all the cyanide into potassium silver cyanide, which compound is neutral to phenol-phthalein, any amount of hydrochloric acid subsequently required to establish neutrality to phenol-phthalein is a measure of the hydroxide and monocarbonate present in the solution.

Further information with respect to the behaviour of cyanides, &c., with indicators of neutrality will be found on page 430.

In the titration with silver nitrate, a slight granular precipitate is generally observed towards the end of the process. This should be disregarded, the end-point being the appearance of a permanent white turbidity which pervades the whole liquid.

Clennell finds that the total cyanogen existing in cyanide liquors free from reducing impurities may be determined very satisfactorily with iodine if the solution be first treated with potassium ferrocyanide. This forms zinc ferrocyanide and potassium cyanide. The liquid is then titrated with iodine, a few drops of dilute starch solution being used as an indicator. Zinc ferrocyanide comes down as a dense white precipitate before the end of the titration, but does not interfere with the observation of the end-point. Solutions containing free hydrocyanic acid must first be neutralised by caustic soda, while such as contain caustic alkali or alkaline carbonate must be neutralised by hydrochloric acid.

Clennell regards the foregoing method as much simpler and equally accurate with that involving precipitation with sodium sulphide, providing that reducing bodies are absent. Unfortunately, the solutions which pass through the zinc boxes are subjected to the powerfully reducing action of nascent hydrogen, which is apt to produce traces of sulphides, sulphites, or thiosulphates, in presence of which the titration with iodine is vitiated.

The cyanide existing as potassium zinc cyanide is regarded by Clennell as valueless as a solvent of gold,[2] and hence it is necessary to supplement the determination of the total cyanide by a process for the determination of the zinc. This can be approximately effected by adding a known excess of standard ferrocyanide solution, acidulating with dilute sulphuric acid, and titrating with standard permanganate. Ferricyanides and cyanides cause no serious interference, but thiocyanates and other reducing agents must be absent. On adding the ferrocyanide no precipitate is formed, but on acidulating the solution the zinc is thrown down as an insoluble ferrocyanide which is unaffected by the permanganate. The difference between the amount of ferrocyanide added and that subsequently found indicates the amount which has reacted with the zinc. The reaction appears to be represented by the following equation :—

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[1] Sodium sulphide is preferable to sulphuretted hydrogen, as its use does not necessitate the previous addition of a large excess of caustic alkali, the presence of which is prejudicial to the accuracy of the subsequent titration with silver solution. Lead carbonate (ordinary white lead) might be advantageously substituted for the litharge employed by Clennell.

[2] This view does not appear to be strictly correct. From experiments made in the laboratory of the African Gold Recovery Company, it appears that pure potassium zinc cyanide, containing no admixture of free potassium

$$3K_2ZnCy_4 + 2K_4FeCy_6 + 12H_2SO_4 = K_2Zn_3(FeCy_6)_2 + 12KHSO_4 + 12HCy.$$

Hence 422 parts of crystallised potassium ferrocyanide correspond to 65 parts of zinc.

In addition to zinc, which, when present to any appreciable extent, renders the results of the direct titration of cyanides either by the silver or the iodine process quite indefinite, Clennell has pointed out the influence of the following substances liable to occur in the cyanide liquors of gold works :—

Ferrocyanides render the results by the silver process somewhat too high, but the error is unimportant, unless the proportion of cyanide is relatively small. With the iodine method the disturbance caused by moderate amounts of ferrocyanides is insignificant.

Ferricyanides interfere very slightly with the correct titration of cyanides either by the silver or by the iodine method. In the former case, instead of the white precipitate of silver cyanide ordinarily formed, a reddish-brown precipitate of silver ferricyanide is produced, which becomes permanent on the completion of the reaction.

Ammonium thiocyanate renders the end-reaction in the silver process somewhat obscure; but this effect appears to be due to the ammonia and not to the thiocyanate. Results above the truth are also obtained in presence of *ammonium carbonate*, but the error may be rectified, as shown by J. S. M'Arthur, by adding potassium iodide, which forms silver iodide, insoluble in solutions of ammoniacal salts.

cyanide, is capable of dissolving gold. Further, soda used in large excess partially decomposes zinc cyanide and potassio-zinc cyanide, with formation of sodium zincate, $ZnNa_2O_2$, and sodium cyanide. This reaction is found to occur in actual practice, an addition of alkali renovating cyanide solutions the solvent action of which approaches exhaustion (W. R. Feldtmann. *Jour. Chem. Soc. Ind.*, 1894, p. 952).

With the iodine process, good results are obtained in presence of ammonium thiocyanate.[1]

W. Bettel (*Chem. News*, lxxii. 286, 298) has published the following ingenious series of processes for the analysis of cyanide liquors. Some of his methods, however, require verification before the results yielded can be fully accepted as accurate.

a. Fifty c.c. of the solution is titrated with silver nitrate to faint opalescence or first indication of a flocculent precipitate. If sufficient ferrocyanide be present to form a flocculent precipitate of zinc ferrocyanide, the result represents the uncombined potassium cyanide, plus cyanide equal to 7·9 per cent. of the potassium zinc cyanide present. *b.* Fifty c.c. of the solution is treated with solution of sodium bicarbonate, free from monocarbonate or excess of carbonic acid, and then titrated with silver nitrate to turbidity. The result, less that obtained in *a*, represents the free hydrocyanic acid. *c.* Fifty c.c. of the solution is treated with excess of normal caustic soda and a few drops of a 10 per cent. solution of potassium iodide. Titration with silver nitrate to opalescence, less the volume required in *a* and *b*, represents the potassium zinc cyanide in terms of potassium cyanide, less 7·9 per cent. Bettel adds:—"A correction is here introduced. The KCy found in *c* is calculated to K_2ZnCy_4. Factor: KCy (as K_2ZnCy_4) $\times 0·9493 = K_2ZnCy_4$. Add to this 7·9 per cent. of total, or for every 92·1 parts of K_2ZnCy_4 add 7·9 parts. If this fraction, calculated back to KCy, be deducted from *a*, we get the true free cyanide (calculated to KCy)." (See page 430.)

Bettel determines ferrocyanides and sulphocyanides by running the cyanide solution into a strongly acidulated solution of $\frac{N}{100}$ potassium permanganate till the colour is just discharged. The ferrocyanide is oxidised to ferricyanide, and the sulphocyanide to hydrocyanic and sulphuric acids. If then a solution of ferric chloride be acidified with sulphuric acid, and 50 c.c. of the cyanide liquor poured in, the ferrocyanide is precipitated as prussian blue. After agitating the liquid it is filtered, and the filtrate titrated with permanganate as before. The result represents the *sulphocyanide* present, and the difference between the two the permanganate required to oxidise the *ferrocyanide*. One c.c. of $\frac{N}{100}$ permanganate oxidises 0·003684 of potassium ferrocyanide, or 0·0001618 gramme of potassium sulphocyanide. In treating spent tailings, or material containing decaying vegetable matter, the liquid should be previously clarified by agitating it with slaked lime. The filtered solution has only a faint straw colour, and is practically free from oxidisable organic matter.

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[1] J. S. C. Wells (*Jour. Soc. Chem. Ind.*, 1896, p. 116) states that the silver method, in presence of free caustic alkali, indicates the total cyanide present, and not simply that existing as cyanide of alkali-metal.

Bettel determines *ferricyanides* by allowing sodium amalgam to act for fifteen minutes on the solution contained in a narrow cylinder, and then determines the ferrocyanide formed by titrating in an acid solution with standard permanganate. After deducting the volume previously found necessary for the oxidation of the ferrocyanide and sulphocyanide, each 1 c.c. of $\frac{N}{100}$ permanganate represents 0·003293 gramme of potassium ferricyanide.

DETERMINATION OF THE METALS IN CYANIDES.

The determination of metals existing in the form or in presence of simple and double cyanides may be effected by the following methods:—

1. All cyanogen compounds without exception (including ferrocyanides, ferricyanides, and cobalticyanides) are completely decomposed, and the metals converted into sulphates or oxides, as the case may be, by treatment in platinum with a mixture of three parts of concentrated sulphuric acid and one part of water. On heating the mixture till nearly all the sulphuric acid is expelled, the residual mass will be obtained free from cyanogen. It may be dissolved in water or acid, and the metals determined by the usual methods. The process is not adapted for the analysis of mercuric cyanide, as some of the metal is volatilised. (When solid, mercuric cyanide may be ignited with soda-lime, and the volatilised mercury collected and weighed.)

2. From solutions containing the cyanide of mercury, silver, or cadmium, the heavy metal may be precipitated by passing sulphuretted hydrogen, and the same process is applicable to solutions of zinc, if sodium or potassium sulphide be substituted for the sulphuretted hydrogen. Nickel, manganese, and copper are not precipitated.

The following method has been largely employed by the author for the determination of the metallic silver in *electro-plating liquors.*

A definite measure of the sample liquid is largely diluted with water, and the whole raised to the boiling point. Sulphuretted hydrogen is passed through the liquid, or ammonium sulphide gradually added. The silver falls as a black sulphide, which is liable to be contaminated with copper and zinc. The washed precipitate is rinsed off the filter into a flask or beaker, and treated with excess of bromine-water, which converts it rapidly and completely into argentic bromide. If any sulphur appears to have separated, a drop of bromine should be added to the residue so as to ensure complete oxidation. Boiling water is now added, and the silver bromide is washed, dried, fused, and weighed. 188 parts by weight of the precipitate represent 108 of metallic silver.

3. For the determination of the precious metal contained in the solution of the double cyanide of gold and potassium used for *electro-gilding*, the author has found the following method very satisfactory:—A measured quantity of the gilding solution is introduced into a porcelain crucible and cautiously concentrated; when in a syrupy condition, a few grammes of pure red-lead or litharge should be added, and the evaporation continued to complete dryness. There is little or no tendency to spitting. The crucible containing the residue is covered and raised for a short time to a moderate red heat. The lead oxide is reduced by the cyanide present, with production of metallic lead and cyanate, and the reduced metal unites with the gold. The resultant button of metal is separated from the slag, and the gold contained in the alloy isolated either by cupellation or by treatment with pure nitric acid.

Electro-silvering solutions may be assayed in a precisely similar manner, but in this case treatment of the rich lead with nitric acid is inadmissible and cupellation *must* be resorted to.

The amount of precious metal found in an electro-depositing liquid is commonly reported in troy ounces, pennyweights, and grains per pint of solution.

4. The determination of the *zinc* in the liquors produced in the cyanide process of treating gold ores may be effected as described on page 439.

Hydrogen Cyanide. Hydrocyanic Acid. Prussic Acid.

HCy; *i.e.*, $H.C{\equiv}N$

Hydrocyanic acid does not appear to occur ready-formed in nature, but it is produced by the action of a peculiar ferment on amygdalin, a glucoside occurring in many plants. Hydrocyanic acid is also produced in many organic reactions, and is formed by the direct union of hydrogen and cyanogen under the influence of the silent electric discharge. It is further formed by the action of the arc-discharge or of a series of electric sparks on a mixture of acetylene and nitrogen.

Anhydrous Hydrocyanic Acid is an intensely poisonous, very volatile liquid, boiling at 26·5° C., and miscible in all proportions with water, alcohol, and ether. It is obtained by passing dry sulphuretted hydrogen gas over dry mercuric cyanide heated to about 30° C. It may also be prepared by heating a mixture of mercuric cyanide and ammonium chloride with strong hydrochloric acid, and passing the gas evolved over fragments of marble and calcium chloride, and then through a tube surrounded by a freezing mixture. The preparation of anhydrous hydrocyanic acid is extremely dangerous, and should be conducted in the open air.

HYDROUS HYDROCYANIC ACID is most conveniently prepared by Wöhler's process, which consists in distilling potassium ferrocyanide with dilute sulphuric acid, when the following reaction occurs:—$2K_4FeCy_6 + 6H_2SO_4 = Fe''K_2FeCy_6 + 6KHSO_4 + 6HCy$.[1]

Dilute hydrocyanic acid of the strength required for medicinal use may be conveniently prepared by decomposing silver cyanide with an equivalent amount of dilute hydrochloric acid. 5 c.c. of hydrochloric acid of 1·163 specific gravity should be mixed with 55 c.c. of distilled water, and the liquid shaken in a stoppered bottle with 6 grammes of silver cyanide. The precipitate is allowed to subside, and the clear liquid poured off. The hydrocyanic acid of the French Codex and United States Pharmacopœia is prepared in this way. Another very convenient plan is to decompose the double cyanide of potassium and zinc (a very stable salt) with tartaric acid. 22 grains of the double cyanide, K_2ZnCy_4, and 49 grains of tartaric acid dissolved in an ounce of water, will yield, on filtration or decantation from the precipitate, a liquid containing 2 per cent. of HCy. Or 9 grammes of tartaric acid may be dissolved in 93 c.c. of water, transferred to a stoppered flask and 4 grammes of potassium cyanide added. The liquid is then well agitated, and decanted when the precipitate has subsided.

Any of these processes will give a product containing 2 per cent. of real hydrocyanic acid. This is the strength of the acid of the Pharmacopœias of Britain, the United States, Germany, Norway,[2] and Switzerland. The hydrocyanic acid of the London Pharmacopœia was of the same strength, but the preparations of the Edinburgh and Dublin Pharmacopœias contained 3·3 per cent. of real acid. "Scheele's acid" is said to have a strength of about 5 per cent.

The strength of the hydrocyanic acid met with in commerce varies to a serious extent. Thus Woodman and Tidy found sixteen samples, sold in one neighbourhood as the B.P. acid, to contain amounts of real hydrocyanic acid ranging from 0·6 to 3·2 per cent. A. Leys (*Pharm. Jour.*, [3], xx. 824) found nine specimens of so-called B.P. acid to range in strength from 1·92 to 0·94 per cent., while the strength of twelve specimens of Scheele's acid examined by R. Wright (*Pharm. Jour.*, [3], xx. 376) varied from 3·6 to 5·7 per cent. of real hydrocyanic acid.

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[1] Seven parts by weight of strong sulphuric acid should be mixed with 14 parts of water. When the liquid is thoroughly cold, it is poured upon 10 parts of coarsely-powdered potassium ferrocyanide contained in a capacious flask, connected with an efficient condensing arrangement terminating in an adapter dipping below the surface of distilled water. If the condenser be inverted, and the vapours dried by passing through a series of tubes filled with calcium chloride and surrounded with water kept at about 30° C., anhydrous hydrocyanic acid may be obtained, and can be condensed by a freezing mixture.

[2] The acid of the Norwegian Pharmacopœia receives an addition of 0·1 per cent. of strong sulphuric acid, as a preservative.

The strength of hydrocyanic acid and preparations containing it can be readily ascertained by one of the modifications of the silver process described on page 431, *et seq.*

Even when only of 2 per cent. strength, hydrocyanic acid is very unstable, being liable to turn brown, especially if exposed to light, with formation of paracyanogen, formic acid, and other products. A production of urea has been observed. Sometimes a solid mass results from the decomposition of the acid.

According to Lescoeur and Rigaut (*Compt. Rend.*, lxxxiv. 310), pure hydrocyanic acid may be preserved, even when anhydrous, for any length of time without alteration; but on addition of a small fragment of potassium cyanide the liquid acquires a brown colour, and in the course of a few days solidifies to a black amorphous mass called "azulmin."

Samples of hydrocyanic acid containing traces of mineral acid are said to keep better than perfectly pure specimens, but the truth of this statement is doubtful.

Very weak hydrocyanic acid, containing 0·1 or 0·2 per cent. of HCy, is much more permanent than the stronger kinds, and can be kept with practically little change. An acid of such a strength is an official preparation in the German Pharmacopœia. The late John Williams observed that hydrocyanic acid, even when of considerable strength, could be kept fairly well if 20 per cent. of glycerin were added to it. Thus a sample containing 37½ per cent. of HCy, 37½ of water, and 25 per cent. of glycerin kept without change for a period of four years.

Dilute hydrocyanic acid is a colourless liquid having an almond-like odour and peculiar bitter taste, both which characters should be observed with the greatest caution, as the acid is extremely poisonous. When boiled, a solution of hydrocyanic acid evolves the gas, which burns when kindled with a beautiful violet flame.

When boiled with concentrated alkalies or mineral acids, hydrocyanic acid suffers hydrolysis with formation of ammonia and formic acid:—$H.CN + 2(H.OH) = H_3N + H.CO.OH$. Conversely, hydrocyanic acid and water are formed when ammonium formate is quickly heated.

Galenical Preparations of Hydrocyanic Acid.

Hydrocyanic acid does not appear to exist ready-formed in the plants yielding it,[1] but is produced by the action of a peculiar ferment called emulsin on the glucoside amygdalin, $C_{20}H_{27}NO_{11}$,[2] which, under its influence and in presence of water, splits up with formation of glucose, benzoic aldehyde, and hydrocyanic acid. (Compare page 90 of this part, and page 19 of Part I.)

Theoretically, 100 parts of amygdalin should yield 5·91 of prussic acid, and the practical results are not far removed from this proportion. The following are the usual proportions of amygdalin contained in, and of prussic acid obtainable from, various natural sources[2] :—

	Amygdalin contained	Yield of HCN.
Bitter-almond pulp, . . .	4¼ per cent.	·25 per cent.
Cherry-stone kernels, . . .	3 ,,	·17 ,,
Wild Service kernels, . . .	1½ ,,	·08 ,,
,, flower, root, and bark,	1 ,,	·06 ,,
Sweet Cassava root,[2] . . .	0·3 ,,	·017 ,,
Bitter Cassava root,[2] . . .	0·45 ,,	·027 ,,

To the presence of hydrocyanic acid the poisonous properties of the essence of cherry-kernels, bitter-almond water (and the crude oil), and laurel-water are due. Good *Kirschwasser* sometimes contains upwards of 1 grain of prussic acid to the pint, and inferior specimens often four times as much.

The following are the usual proportions of real hydrocyanic acid present in certain preparations containing it :—

Crude bitter-almond oil, ...	8 to 15 per cent.
Bitter-almond water, ...	¼ to 1 ,,
Cherry-laurel oil, ...	2 to 3 ,,
Cherry-laurel water, ...	¼ to ¾ ,,
Cluster cherry oil, ...	9 to 10 ,,

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[1] The roots of both sweet and bitter cassava are stated to contain ready-formed hydrocyanic acid.

[2] Amydalin has been found in the seeds, leaves, flowers, and in some cases in the bark, of most species of the sub-orders *Amydaleæ* and *Pomeæ* (order, *Rosaceæ*), including the apple, pear, quince, sloe, bullace, plum, damson, almond, peach, nectarine, apricot, cherry, hawthorn, mountain ash, etc.

Although in medico-legal investigations the possible introduction of hydrocyanic acid into the body from natural sources should not be overlooked, the importance of such sources of error is generally much over-rated. In the trial of Tawell (1845) for murdering Sarah Hart, an expert was pressed, in the witness-box, to say whether apple-pips did not contain prussic acid, and, if so, whether the fact of their having been found in the stomach of the deceased might not account for her death. After expressing his inability to state the exact amount of prussic acid obtainable from apple-pips, and being encouraged to express his opinion as to the quantity of pips requisite to yield a fatal dose of the poison, he caused the collapse of Fitzroy Kelly, the prisoner's counsel, by opining that "about a peck" might suffice.

Leger (*Pharm. Jour.*, [3], iii. 971) found the proportion of hydrocyanic acid to vary considerably in laurel-water prepared in a similar manner from leaves gathered at different seasons, the extreme figures being 0·044 and 0·125 gramme in the 1500 grammes of distillate obtained from 1000 grammes of leaves. These results show a far smaller proportion of hydrocyanic acid than those previously reported.

Perinelle found that cherry-laurel water prepared in May contained only 0·039 per cent. of hydrocyanic acid, while that made in November contained 0·134 per cent.

In the case of all these liquids, the proportion of hydrocyanic acid, and, consequently, the poisonous effects, become greatly diminished by long keeping. The oils may be assayed for hydrocyanic acid by the methods described under "benzoic aldehyde" (Part i. page 20), and the aqueous liquids as described on page 431 (of this part).

In order to avoid the uncertainty which attends the use of bitter-almond water, cherry-laurel water, and other preparations containing hydrocyanic acid, **Hermes** has proposed to substitute **chloral cyanhydrate**, $CCl_3.CH(OH).CN$. This compound forms a crystalline powder, readily soluble in water, alcohol, and ether. It volatilises somewhat in a current of steam, but is split up by the treatment into chloral and hydrocyanic acid. Alkalies also decompose it with formation of a cyanide. Chloral cyanhydrate is very stable, and aqueous solutions remain for a long time unaltered. It has been ascertained to exercise the same physiological action as pure hydrocyanic acid.

Toxicology of Hydrocyanic Acid.

Hydrocyanic acid, in any form, and however administered, is an intensely active poison to every animal, with the exception of the frog.[1] When swallowed, injected, inhaled, and sometimes even when merely applied to the sound skin, and, according to **N. Gréhant**, when applied to the mucous membrane of the eye, its action is energetic. An internal dose of three to four grains of real hydrocyanic acid is generally fatal to the human subject, and less than 1 grain has been known to cause death.

Chlorine and ammonia appear to act as antidotes in cases of prussic acid poisoning, but their mode of action is obscure, since the presumed products of their action, namely cyanogen chloride and ammonium cyanide, are as poisonous as hydrocyanic acid itself.

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[1] According to W. H. **Broadbent** (*Pharm. Jour.*, [3], xxi. 136) the frog becomes gradually narcotised by the inhalation of hydrocyanic acid gas, but recovers when brought into the open air.

The detection of hydrocyanic acid in the body is rendered difficult by the great facility with which the acid decomposes.[1]

On opening the stomach and intestines the odour of hydrocyanic acid is often perceptible. These viscera are often quite natural in appearance, but sometimes more or less inflamed and congested. The lungs, liver, spleen, and kidneys are always found gorged with blood. The venous system is invariably gorged with blood, the arteries being empty. The blood has undergone change; it may be black or oily, or of a cochineal-red colour. It often smells of the poison, which may frequently be distilled from it.

To detect hydrocyanic acid in the contents of a stomach the analyst should proceed as follows:—

Note the reaction of the liquid portion. If not distinctly alkaline to litmus, the poison (if present) was probably administered as free hydrocyanic acid, and not as cyanide of potassium. (The various double cyanides used for electro-deposition of metals have a neutral reaction in the absence of excess of potassium cyanide.)

Stir up the stomach and its contents with cold water, and introduce the thick liquid into a flask adapted to a Liebig's condenser, allowing the end of the condensing tube to be immersed in a small quantity of water. Apply a moderate heat to the flask (best by an external bath of salt water), and distil over about half the liquid. To the distillate apply carefully tests 2, 3, and 4 on pages 428, 429. It is preferable to avoid any addition of acid to the liquid to be examined, as the saliva contains traces of thiocyanates (sulphocyanides), which might possibly yield traces of hydrocyanic acid on distillation with a mineral acid. If the distillate has given negative results when tested for prussic acid, continue the distillation after rendering the contents of the flask distinctly acid with tartaric acid. If hydrocyanic acid be now found in the distillate, the poison must have been present as a readily decomposable cyanide. As a preliminary test, A. Seyda recommends the guaiacum-copper reaction (page 430). If the distillate gives no indication of hydrocyanic acid by this test, he considers it useless to proceed further. But if an affirmative result is obtained, other tests should be employed. If only a small quantity of hydrocyanic acid be present in the distillate, it is most conveniently detected by precipitating it by silver nitrate, and applying the iron and sulphur tests to the precipitate (page 428).

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[1] Reichart mentions a case in which he succeeded in detecting hydrocyanic acid two months after death. He obtained no definite results from the urine in this case, but the distillate from the organs previously acidulated with tartaric acid yielded affirmative results both by the guaiacum and the prussian-blue tests.

C. Bischoff (*Ber.*, xvi. 1337; abst. *Jour. Chem. Soc.*, 1883, page 1022) has published a number of results showing the capability of absorption of hydrocyanic acid by various organs of the body.

Before finally concluding that all metallic cyanides are absent, it is desirable to repeat the distillation after adding a considerable excess of moderately dilute sulphuric and hydrochloric acids. Ferrocyanides, ferricyanides, and mercuric cyanide will in this case be decomposed. Hence the absence of ready-formed ferrocyanides or ferricyanides should be previously ascertained by testing portions of the acidulated contents of the stomach with solutions of ferric and ferrous salts.

H. Beckurts (*Chem. News*, xlviii. 199), W. J. Taylor (*Chem. News*, l. 227), and W. Autenreith (*Arch. Pharm.*, ccxxxi. 99) have pointed out that potassium ferrocyanide is less stable in presence of dilute acids than is generally supposed. Carbon dioxide at 74° liberates hydrocyanic acid from it, with precipitation of potassium ferrous ferrocyanide, K_2Fe_2Cfy, and casein, peptone, and artificial digestive fluid exercise a similar action at 40° C. Autenreith considers, therefore, that the only certain way to detect hydrocyanic acid or simple cyanides in presence of ferrocyanides is by Jacquemin's process. He distils the material with a considerable amount of sodium bicarbonate, and examines the distillate for hydrocyanic acid. If the presence of mercuric cyanide be suspected, sulphuretted hydrogen water must be also added, as the bicarbonate does not itself decompose mercuric cyanide. Or mercuric cyanide may be decomposed by adding a few bright strips of zinc to the liquid before commencing the distillation. Ferricyanides, thiocyanates, sulphates, and ammonium salts do not interfere with the process.

Mercuric cyanide may be decomposed by distillation with hydrochloric acid. P. Plugge recommends a mixture of sodium chloride and oxalic acid for the purpose, while S. Lopes (abst. *Jour. Chem. Soc.*, 1893, ii. 502) suggests that, after testing for other simple cyanides, mercuric cyanide should be sought for by distillation with sodium bicarbonate, by acidulating the mixture with tartaric acid, adding ammonium chloride in excess, and again distilling. A double chloride of mercury and ammonium is said to be formed, and hydrocyanic acid distils with the steam. To test for cyanides in presence of ferrocyanides, Lopes heats the substance to 100° with milk of lime, in order to decompose ammonium salts, which, if present, may react with the ferrocyanide to form volatile ammonium cyanide. When all the ammonia has been driven off, the solution is filtered and distilled with excess of sodium bicarbonate, as recommended by Autenreith.

Barfoed's process for the detection of hydrocyanic acid and poisonous cyanides in presence of ferrocyanides is based on the fact that ether extracts hydrocyanic acid, but not hydroferrocyanic acid, from an aqueous liquid. Hence the material to be tested is acidulated with tartaric acid, and shaken several times with an equal volume of ether. The united ethereal extracts are agitated with dilute solution of soda, which is separated and tested for hydrocyanic acid in the usual manner.

A. **Vogel** has proposed the reduction of picric acid to picramic acid as a test for hydrocyanic acid. The reaction is very delicate, but as it is produced by a great many other reducing substances, its value is limited.

G. **Portmann** (*Jour. Soc. Chem. Ind.*, v. 679) gives the following test for the detection of minute quantities of hydrocyanic acid. It is based on the formation of a nitroprusside by the action of a metallic nitrite on potassium cyanide in presence of a ferrous salt. The liquid to be tested is treated with a few drops of a solution of potassium nitrite, two or three drops of ferrous sulphate solution, and enough dilute sulphuric acid added to cause the yellow-brown colour of the basic ferric salt to become a light yellow. The liquid is then boiled, cooled, the excess of iron precipitated with ammonia, and the solution filtered. A few drops of freshly prepared ammonium sulphide are added, when, if any hydrocyanic acid were present in the original liquid, a violet coloration immediately appears, which changes rapidly through blue and green to a yellow colour. Very minute traces of hydrocyanic acid produce a bluish-green colour, rapidly changing to greenish-yellow. Portmann estimates the delicacy of this test at 0·00003 gramme hydrocyanic acid dissolved in 10 c.c. of water (1 : 312,000).

One of the main causes of the disappearance of hydrocyanic acid in the dead body is its reaction with ammonium sulphide, and probably with other sulphuretted bodies produced by putrefaction, whereby thiocyanates (sulphocyanides) are formed. Therefore, where hydrocyanic acid is to be sought for a considerable time after death, it may sometimes be detected by rendering the materials distinctly, but not excessively, alkaline with caustic potash, and then adding excess of alcohol. The liquid is filtered, and evaporated to dryness. The residue is redissolved in water, acidulated with hydrochloric acid, and ferric chloride added, when a red colour will be produced if a thiocyanate were present in the stomach.

In applying this test it must not be overlooked that thiocyanates are normally present in the saliva. Hence they exist in traces in the stomach, and can be detected therein by ferric chloride. The presence of thiocyanates is a source of error in Uffelmann's reaction for lactic acid in gastric juice (page 419).

A mixture of solution of ferrous sulphate with alkali is sometimes given as an antidote in cases of poisoning by prussic acid. In such cases, prussian blue will be formed on acidulating the contents of the stomach with hydrochloric acid.

Metallic Cyanides.

The simple metallic cyanides are typified by potassium cyanide. Much ingenuity has recently been exercised with a view to their economical production, since their application in the metallurgy of gold has enormously increased the demand during the last few years, and has given a great impetus to invention. Hence, numerous patents have been obtained with a view of manufacturing cyanides by cheaper and simpler processes than those which sufficed when the demand was comparatively small.[1]

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[1] Numerous attempts have been made to manufacture metallic cyanides synthetically, without the use of animal refuse as raw material. These processes may be classed as (1) those in which ammonia was the source of nitrogen (these being similar in principle to the old prussiate process); (2) methods in which it was sought to utilise atmospheric nitrogen; and (3) processes based on the desulphurisation of sulphocyanides.

According to the process of Hood and Salamon (*Eng. Patent*, No. 5354, 1891), ammonia, carbon disulphide, and washed Weldon mud (calcium manganite) are heated together in closed vessels to 100° or somewhat higher, whereby manganese sulphocyanide and sulphide, free sulphur, and water are formed. Slaked lime or other base may be added in quantity sufficient to react with the sulphocyanide, when on washing the resultant mass calcium sulphocyanide goes into solution, while manganese sulphide and sulphur remain undissolved and may be revivified by exposure to air. In the modified process of Crowther and Rossiter (*Eng. Patent*, No. 17,845, 1893), calcium sulphocyanide is obtained by digesting carbon disulphide with ammonia and lime in quantities slightly in excess of those required by the reaction:—$2CS_2+2NH_3+2CaH_2O_2=Ca(SCN)_2+CaH_2S_2+4H_2O$. The product is treated with a current of carbon dioxide (preferably lime-kiln gases), as in Chance's sulphur-recovery process, whereby sulphuretted hydrogen is evolved (and may be utilised), calcium carbonate is precipitated, and sulphocyanide remains in solution. In this process, sulphides may be substituted for hydroxides, the reaction in the case of barium sulphide being:—$2CS_2+2NH_3+4BaS=Ba(SCN)_2+3BaH_2S_2$. (*N.B.*—This reaction affords a means of utilising the sulphur of barium sulphate.—A. H. A.)

The calcium sulphocyanide obtained by the above methods can be converted into the potassium salt by treatment with potassium sulphate. The product

POTASSIUM CYANIDE. KCy, *i.e.*, K.C≡N

Potassium cyanide is a product of the first commercial importance. It has long been employed in the electro-deposition of metals, in photography, for cleaning silver, &c.

A cyanide always results from the ignition of nitrogenous organic matter with caustic or carbonated alkali, but until recently almost the only process for preparing potassium cyanide which has been found commercially available has consisted in the ignition of a mixture of dry potassium ferrocyanide and potassium carbonate to dull redness, when the following reaction occurs:—

$$K_4FeCy_6 + K_2CO_3 = KCyO + 5KCy + Fe + CO_2.$$

In order to obtain a good yield or a product of high quality it is essential to observe a number of precautions, some of which are kept jealously secret.

The product prepared by this process always contains cyanate, and usually carbonate. Sulphide is also present if the alkali used contained any sulphate. By heating potassium ferrocyanide alone, or by adding charcoal or sodium to the mixture, the formation of cyanate may be avoided. By replacing the potassium

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is desulphurised by heating with metallic iron, lead, or zinc. Playfair employs sodium sulphocyanide instead of the potassium salt. This he desulphurises in graphite vessels by melting it with zinc and powdered carbon (see *Eng. Patents*, Nos. 7764 of 1890 and 8669 of 1895).

The British Cyanides Company, Limited, now manufacture cyanides extensively near Birmingham by the Hood-Salamon and Crowther-Rossiter processes. The Playfair patent is owned by the same company, and is employed in conjunction with the above processes. Cyanide of zinc is employed for removing alkaline sulphides from the product.

A more recent process of obtaining cyanides has been proposed by Hood and Salamon (*Eng. Patent*, 21,239, 1893), who heat a mixture of sodium carbonate or bicarbonate with a reducing agent such as metallic zinc, manganese, or lead in a current of ammonia, when the following reaction takes place:—$NaCO_3 + NH_3 + Zn = NaCN + NaHO + H_2O + ZnO$.

A better result is obtained by passing carbon dioxide gas together with the ammonia, and by substituting a mixture of oxide of zinc, lead, or manganese with carbon for the corresponding metal.

When heated to redness, trimethylamine is decomposed with formation of ammonium and hydrogen cyanides and hydrocarbons. Upon this fact a process has been devised for the manufacture of cyanides from the trimethylamine from beetroot molasses, and is said to be in use at the works of the Croix Company.

The synthetical processes of manufacturing cyanides form the subject of an interesting paper by N. Caro (*Jour. Soc. Chem. Ind.*, 1896, p. 33). J. T. Conroy has recently published the results of various suggestive experiments on the production of cyanides (*Jour. Soc. Chem. Ind.*, 1896, p. 8).

carbonate by sodium carbonate, a product containing a higher percentage of cyanogen is obtained, owing to the lower combining weight of sodium; and by substituting sodium ferrocyanide for the potassium salt, a product is obtainable which contains cyanogen equivalent to 100 per cent., or even more, of potassium cyanide. 100 per cent. potassium cyanate is now manufactured by Erlenmeyer's process, in which potassium ferrocyanide is heated with metallic sodium:—$K_4FeCy_6+Na_2=4KCy+2NaCy+Fe$.

Potassium cyanide fuses at a low red heat to a transparent liquid, which is a most powerful reducing agent, liberating tin, antimony, bismuth, lead, &c., from their oxides and sulphides, with formation of potassium cyanate or thiocyanate. The thiocyanate also results from fusing the cyanide with free sulphur or a thiosulphate (hyposulphite), or by boiling the aqueous solution with sulphur.

Potassium cyanide seems to volatilise unaltered at a white heat. It is frequently formed in considerable quantity in blast-furnaces. When heated in the air it forms cyanate, and in presence of vapour of water potassium carbonate and ammonia result.

By the action of potassium permanganate in presence of caustic alkali, on a cold solution of potassium cyanide, cyanate is formed (J. Volhard, *Annalen*, cclix. 377).

Cyanide of potassium generally occurs in commerce in milk-white cakes or rods, but it may be obtained in cubes by crystallisation from hot alcohol. It is very deliquescent and soluble in water, forming an alkaline and intensely poisonous liquid. Potassium cyanide may be kept unaltered in close vessels, but in the air it absorbs carbonic acid with liberation of hydrocyanic acid. By passing a stream of carbonic acid gas through the aqueous solution the cyanide may be completely decomposed. On boiling the aqueous solution, potassium formate and ammonia are gradually produced:—$KCN+2H_2O=KCHO_2+H_3N$.

Potassium cyanide unites with most of the cyanides of the heavy metals forming more or less stable double cyanides much used in electro-metallurgy. The methods of assaying and analysing these bodies have been already described (pages 435 to 442). Owing to the strong tendency which exists to form a double cyanide of potassium and silver, potassium cyanide dissolves all silver compounds except the sulphide. Hence its employment as a fixing agent in photography.

Commercial cyanide of potassium is most conveniently assayed for the proportion of real cyanide present by Liebig's silver process described on page 432. The results are not affected by the ordinary impurities of commercial cyanide, with the exception of soluble sulphides, which must be removed previous to titration. The process is carried out as follows:—6·510 grammes (=100·5 grains) of the powdered sample are treated in a 500 c.c. flask with about 300 c.c. of cold water. When solution is complete, about 1 gramme of lead carbonate (white lead) should be added, and the flask strongly agitated to convert any soluble sulphides into sulphide of lead. The solution is now diluted to 500 c.c., well agitated, and passed through a dry filter. 100 c.c. of the filtrate is then treated with a few drops of a solution of potassium iodide, and titrated with decinormal silver nitrate, which is added until a white turbidity or milkiness is produced, which is permanent after agitation. The flask containing the solution should be placed on a black surface. When the above quantities are employed, each c.c. of the decinormal silver solution corresponds to 1 per cent. of cyanide, expressed in terms of potassium cyanide.

By the substitution of sodium carbonate for the potassium salt, an article is now prepared which shows on assay from 98 to 100 per cent. of "potassium cyanide," and by employing sodium ferrocyanide a product even richer in cyanide is obtainable.[1]

The potassium cyanide required for electro-gilding should contain 90 per cent. of real KCy. For electro-plating liquids 70 per cent. cyanide can be used, whilst for photographic purposes a less pure sample than the latter will suffice.

Owing to its manner of preparation, and to the materials employed, commercial potassium cyanide contains many impurities, the chief of which are:—Potassium cyanate, potassium thiocyanate, potassium ferrocyanide, potassium formate, potassium chloride, potassium carbonate, potassium silicate, potassium sulphate, and potassium sulphide. In addition to these compounds of potassium (and sodium), commercial cyanide may contain more or less alumina, calcium compounds, &c., and in old damp cyanide ammonia is frequently present. The presence or absence of some of the above impurities will depend upon the quality of materials used, the employment of damp materials, the free admission of air, or the use of an unsuitable temperature.

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[1] T. B. Stillman found 16·90 per cent. of NaCy and 82·83 of KCy in a sample of commercial "98 per cent. cyanide." The alkali-metals were determined by evaporating the solution of the sample with excess of sulphuric acid and igniting and weighing the resultant sulphates. The sulphate was then determined by precipitation with barium chloride, and the potassium and sodium calculated from the data obtained. To their weights were added that of the cyanogen, as determined by silver solution.

It has been pointed out by R. Kayser (*Chem. Zeit.*, 1892, xvi. 1148) that a so-called "100 per cent." potassium cyanide may contain as much as 15 per cent. of impurities if the potassium be replaced by sodium. He also states that if an article containing sodium cyanide be employed in an electro-gilding bath for dissolving gold electrolytically, trouble arises from the deposition of the difficultly soluble sodium aurous cyanide on the anode. The author is not aware of any such difficulties occurring in the electro-gilding works of Sheffield.

The above impurities may be detected in the following manner:—

Potassium cyanate will dissolve in alcohol of specific gravity 0·849, and this solution, on addition of hydrochloric acid, will evolve carbon dioxide. Or, on adding water to the alcoholic solution, and boiling off the alcohol, the liquid will give a precipitate of calcium carbonate with calcium chloride (see also page 484). Cyanate may also be detected by the following application of Blomstrand's colour-reaction:—A strong solution of the sample is decomposed by passing carbon dioxide through it until no more hydrocyanic acid is evolved. By these means E. A. Schneider (*Jour. Soc. Chem. Ind.*, 1895, p. 887) found that 3 grammes of potassium cyanide were decomposed in forty-five minutes. To the resulting liquid Schneider adds sufficient 95 per cent. alcohol to precipitate the potassium carbonate formed. The filtrate is then slightly acidified with acetic acid, and some cobalt acetate solution added. An intense blue colour, due to the formation of the double cyanate of cobalt and potassium, is produced, which renders easy the detection of as little as 0·35 per cent. of cyanate. If present in smaller quantities, more of the cyanide must be taken, dissolved in the smallest possible quantity of water, and the greater part of the cyanide precipitated by the addition of absolute alcohol. The filtrate is then treated with carbon dioxide, and tested as before.

Chlorides may be detected as described on page 428, and can be determined by Siebold's volumetric method (page 434), or gravimetrically, as on page 431.

Formates, if present, will cause the salt to blacken on ignition. They may be detected more certainly by precipitating the cold dilute solution of the sample with excess of silver nitrate solution, filtering cold and heating the clear liquid. In presence of a formate, metallic silver will be precipitated. The filtrate from the precipitate produced by silver nitrate will also give a red colour with ferric nitrate or sulphate if a formate be present.

Carbonates will remain insoluble on treating the sample with hot alcohol of 0·849 specific gravity.

Silicates can be detected and estimated in the ordinary way by evaporation to dryness with hydrochloric acid, the residue insoluble in acidulated water being silica.

Sulphates are detected by the formation of a white precipitate on adding barium chloride to a solution of the sample previously acidulated by hydrochloric acid.

Sulphides will give a black precipitate with mercuric chloride, and a yellow precipitate with a solution of cadmium. They can be separated by agitating the solution with lead carbonate.

Free ammonia can be recognised by the smell, and determined by treating the solution with an alkaline solution of sodium hypobromite and measuring the nitrogen gas evolved (see page 277).

MERCURIC CYANIDE. $HgCy_2$.

Mercuric cyanide is almost the only simple cyanide of a heavy metal which is soluble in water. Owing to its stability it reacts in an anomalous manner. Thus, it does not respond to the iron tests for cyanides (No. 3, page 429), and is not precipitated by silver nitrate. It yields, however, a yellowish-white precipitate of palladious cyanide on addition of palladious nitrate. Mercuric cyanide is not precipitated by alkalies, but by boiling with hydrochloric acid hydrocyanic acid is evolved and mercuric chloride formed. Solution of mercuric cyanide is readily decomposed by sulphuretted hydrogen, and, after separation from the precipitated mercuric sulphide, the cyanide in the liquid can readily be detected by Liebig's test (No. 4, page 429). Mercuric cyanide may also be decomposed by digesting the solution with metallic cadmium, which precipitates metallic mercury and forms cadmium cyanide, $CdCy_2$, in which the cyanogen is readily determined.

Owing to the tendency to form mercuric cyanide, many simple and double cyanides are decomposed by boiling with yellow mercuric oxide and water. This is true of ferrocyanide and ferricyanide of potassium and also of prussian blue, but not of cobalticyanides.

No mercurous cyanide is known; on adding mercurous nitrate to a liquid containing hydrocyanic acid, or a metallic cyanide, metallic mercury separates, and soluble mercuric cyanide is formed. A similar reaction occurs on treating calomel with excess of hydrocyanic acid (*Pharm. Jour.*, [3], vi. 801, 818; xx. 792, 828; xxii. 1002).

When dry mercuric cyanide is heated it decomposes with formation of metallic mercury, paracyanogen, and cyanogen gas.

A hot solution of mercuric cyanide readily dissolves yellow mercuric oxide. The resultant solution has been recommended instead of solid mercuric oxide for the separation of cobalt from nickel (compare page 479).

A solution of mercuric cyanide to which caustic alkali has been added has been recommended by Knapp, in place of Fehling's solution, for the titration of glucose, and by H. W. Wiley for oxidising and destroying dextrose and maltose while leaving dextrin unchanged. J. A. Wilson, however, finds the distinction in these cases is not sufficiently sharp to serve as a foundation for a quantitative process (*Chem. News*, lxv. 169).

DOUBLE CYANIDES.

As already stated, the cyanides of the heavy metals exhibit a remarkable tendency to form double salts[1] which in many instances are of an exceedingly stable nature, so that in some cases neither the cyanogen nor the heavy metal is recognisable by any reaction which does not involve actual destruction of the compound cyanide.

Such stable double cyanides are not precipitated by alkalies or decomposed by carbonic acid, and their aqueous solutions are usually quite neutral in reaction. On ignition without access of air they are decomposed, and the cyanide of the alkali-metal may then be dissolved out from the residual heavy metal or metallic carbide by means of water.

Many of the double cyanides are decomposed on addition of a dilute mineral acid, as in the following instance:—

$$KAgCy_2 + HNO_3 = AgCy + HCy + KNO_3.$$

The liberated hydrocyanic acid has, in such cases, no tendency to combine with the cyanide of the heavy metal.

Double cyanides which suffer decomposition in the above manner respond to the tests for simple cyanides (page 428), except that the precipitate produced by silver nitrate in a neutral solution is not pure argentic cyanide, but a mixture or compound of the two cyanides of the heavy metals, as in the following instance: — $2KCy, ZnCy_2 + 2AgNO_3 = 2AgCy, ZnCy_2 + 2KNO_3$. On treating the precipitate with dilute nitric acid, the zinc cyanide dissolves and argentic cyanide remains.

Of the readily decomposable double cyanides, those of mercury, silver, zinc, and cadmium are decomposed by sulphuretted hydro-

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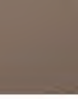
[1] The cyanides are commonly represented as having the constitution of nitriles, M.CN; but E. Divers (*Jour. Chem. Soc.*, xlvii. 227, xlix. 582) has shown that there are good reasons for regarding them as carbamines, M.NC. He further suggests that by attributing to the cyanides a constitution half carbamine, half nitrile, an explanation is afforded of their remarkable tendency to form double salts. Thus:—
Potassium cyanide, K.N:C:N:C.K. Silver potassium cyanide, Ag.N:C:N:C.K.
Hence free cyanogen would probably have the following constitution:—



ly and completely, with precipitation of the correspond- llic sulphides. Most of the other double cyanides of (*e.g.*, copper, nickel) are decomposed very imperfectly, all.

f the double cyanides are of a still more stable character, eatment with a dilute mineral acid, the liberated hydro- d remains in close combination with the cyanide of the etal forming a new compound acid giving rise to a and characteristic series of salts. The following equa- esent the action of hydrochloric acid on (*a*) potassio- yanide, and (*b*) potassio-cobaltic cyanide:—

$y_6 + 4HCl = 4KCl + H_4FeCy_6$ (Hydrogen ferrocyanide).

$y_6 + 3HCl = 3KCl + H_3CoCy_6$ (Hydrogen cobalticyanide).

llowing are the chief classes of stable double cyanides:—

ides, . .	$4MCy,FeCy_2$; *i.e.*, M_4FeCy_6 or M_4Cfy''''.
ides, . .	$3MCy,FeCy_3$; *i.e.*, M_3FeCy_6 or M_3Cfdy'''.
rricyanides russides),	$2MCy,FeCy_3NO$; *i.e.*, M_2FeCy_5NO.
anides,[1] .	$3MCy,CoCy_3$; *i.e.*, M_3CoCy_6.
anides,[1] .	$3MCy,CrCy_3$; *i.e.*, M_3CrCy_6.
anides, .	$2MCy,PtCy_2$; *i.e.*, M_2PtCy_4.

the foregoing description it is evident that the double may be conveniently arranged in two classes, namely: which are readily decomposed by dilute mineral acids, hose which are not materially affected by such treatment. important members of Class 1 are described below, while ipal stable double cyanides (Class 2) are considered ly in separate sections (see ferrocyanides, ferricyanides, nides, &c.).

ily Decomposable Double Cyanides.

compounds have a great practical interest from their ap- in the treatment of gold ores and in electro-metallurgy. mination of the metals contained in them is described on and of the cyanide on pages 434 and 437.

IUM ZINC CYANIDE. $2KCy,ZnCy_2$.

alt is the type of the readily-decomposable double It has a practical interest as a convenient source of free

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paration and reactions of cobaltocyanides, chromocyanides, man- es, and manganocyanides has been described by Descamps . *Chem. Soc.*, xlii. 154), by O. T. Christensen (abst. *Jour.* xlviii. 737), and by H. Moissan (*ibid.*, p. 738).

hydrocyanic acid (page 443), as a suitable compound for the electro-deposition of zinc, and as a constituent of the cyanide liquors of gold-extraction works.

Potassium zinc cyanide is readily prepared by precipitating a solution of zinc sulphate or chloride with an equivalent amount of potassium cyanide, and dissolving the washed precipitate of zinc cyanide in a solution of a second equivalent of potassium cyanide. On concentration, the solution deposits large, colourless, regular octahedra of the double cyanide. The salt is fusible, permanent in the air, and very soluble in water to form a solution of a sweet taste. Addition of a moderate quantity of acetic, hydrochloric, or sulphuric acid to the solution precipitates zinc cyanide, which redissolves in excess of the precipitant.

The solution of potassium zinc cyanide has an alkaline reaction to litmus. With methyl-orange the neutral point is stated to correspond with the complete conversion of the potassium and zinc into chlorides : — $K_2ZnCy_4 + 4HCl = 2KCl + ZnCl_2 + 4HCy$. With phenol-phthaleïn the neutral point is reached, according to W. Bettel, when sufficient cyanide has been added for the reaction :—

$$(K_2ZnCy_4)_{10} + 3HCl = 3HCy + 3KCl + (ZnCy_2)_{10},(KCy)_{17}.$$

Upon this reaction W. Bettel has based a process for the analysis of the cyanide liquors of gold works (see page 440).

Bettel states that an exactly parallel reaction occurs with silver nitrate, the reaction in this case being :—

$$(K_2ZnCy_4)_{20} + 3AgNO_3 = 3KAgCy_2 + 3KNO_3 + 2[(ZnCy_2)_{10},(KCy)_{17}]$$

On addition of excess of caustic potash to the solution of potassium zinc cyanide, potassium cyanide and zincate are formed according to the equation :—

$$K_2ZnCy_4 + 4KHO = 4KCy + ZnK_2O_2 + 2H_2O.$$

Caustic soda acts similarly, as also do potassium and sodium monocarbonates, but bicarbonates have no action.

Sodium sulphide or sulphuretted hydrogen throws down the whole of the zinc from a solution of potassium zinc cyanide. This reaction affords a means of separating zinc from nickel and copper, the sulphides of which are not similarly precipitated.

The zinc contained in the cyanide liquors of gold works may also be determined with moderate accuracy in the manner described on page 439.

POTASSIUM COPPER CYANIDE.

When to a solution of cupric sulphate potassium cyanide is added in limited quantity, a yellowish-brown precipitate of cupric cyanide, $CuCy_2$, is obtained. On boiling the liquid this suffers more or less complete reduction to white cuprous cyanide, Cu_2Cy_2, cyanogen being evolved as gas. Cuprous cyanide dissolves in potassium cyanide solution to form a colourless liquid containing potassium cuprous cyanide, $2KCy,Cu_2Cy_2$, which can be obtained in colourless crystals on evaporation. The solution is not precipitated by alkalies nor by sulphuretted hydrogen, a fact utilised in analysis to separate copper from cadmium.

A solution of ammonio-cupric sulphate becomes colourless or faintly yellow on addition of potassium cyanide, a fact on which is based Parkes' process for the volumetric determination of copper (see also G. Denigès, *Analyst*, June 1896).

The copper is not precipitated from a solution of potassium copper cyanide by contact with iron, a fact which is utilised in the electro-deposition of copper on iron surfaces.

If potassium cyanide be added to boiling Fehling's copper solution, the blue colour is destroyed as in the last case, and the liquid gives no precipitate of cuprous oxide when boiled with glucose; but if Fehling's solution be present in quantity more than sufficient to react with the potassium cyanide used (that is, if the mixed solutions retain a blue colour), this extra portion will suffer reduction by glucose; but instead of the cuprous oxide being precipitated it will remain in solution, and the progress and end of the reduction will be indicated by the gradual lessening and ultimate entire disappearance of the blue colour. This reaction is utilised in Gerrard's cyano-cupric process of glucose titration (see Allen's *Chemistry of Urine*, page 74).

POTASSIUM SILVER CYANIDE. $KCy,AgCy$.

This compound is obtained by precipitating silver nitrate by an equivalent amount of potassium cyanide, and dissolving the white curdy silver cyanide thus obtained in a second equivalent of potassium cyanide solution. On evaporation, the solution deposits hexagonal tables of potassium silver cyanide. The compound may also be obtained by the action of potassium cyanide solution on metallic silver in presence of air, by dissolving silver chloride in potassium cyanide solution, or by adding potassium cyanide to silver nitrate solution until the precipitate at first formed is redissolved (compare page 432).

A solution of potassium silver cyanide is decomposed on addition of a dilute mineral acid with evolution of hydrocyanic acid and precipitation of silver cyanide. From a *dilute* solution the silver is completely thrown down by sulphuretted hydrogen or ammonium sulphide. Any zinc present will accompany the silver, but nickel and copper will remain in solution.

Potassium silver cyanide is extensively used for electro-plating.

The contained silver may be determined as on page 441, and the cyanogen as described on page 435.

POTASSIUM GOLD CYANIDES.

On adding potassium cyanide to a solution of auric chloride, a precipitate of auric cyanide, $AuCy_3$, is first obtained, but this dissolves with great facility in excess of the reagent with formation of potassium auric cyanide, $KCy,AuCy_3$, which on evaporation can be obtained in colourless tables containing 1 aqua. The solution is used for electro-gilding.

A solution of potassium cyanide readily dissolves gold if access of air be permitted, the reaction apparently being:—

$$2Au_2 + 8KCy + 2H_2O + O_2 = 4(AuCy,KCy) + 4KHO.$$

On evaporation the solution yields colourless octahedral crystals of potassium aurous cyanide.

The foregoing reaction is the foundation of the cyanide process of gold extraction now so extensively used for the treatment of South African ores. The quantity of cyanide theoretically necessary to dissolve a given weight of gold is very small in comparison with the weight required in practice,[1] which is at least 40 parts of

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[1] This is due in the first place to the instability of potassium cyanide, which is decomposed by atmospheric carbon dioxide with formation of hydrocyanic acid and potassium carbonate. Further loss takes place by oxidation of the cyanide to cyanate and carbonate, and in presence of excess of caustic alkali much loss results from hydrolysis with formation of potassium formate and ammonia. More or less decomposition of the cyanide also probably occurs in dilute solutions, with formation of hydrocyanic acid and potash:— $KCy + H_2O = HCy + KHO$. Evidence of this reaction is afforded by the fact that on passing nitrogen or other inert gas through a cold dilute solution of potassium cyanide, hydrocyanic acid is volatilised, and the fact accounts for the odour of hydrocyanic acid always perceptible in the vicinity of cyanide tanks freely exposed to the air. A further loss of cyanide occurs from the formation of ferrocyanides and analogous compounds. The appearance of a blue coloration on the surface of the tailings or in the cyanide solution is a certain indication that acid salts of iron are present, and that a large loss of cyanide has occurred.

Traces of free sulphuric acid exist in pyritous tailings, and cause evolution of hydrocyanic acid.

Ferrous sulphate reacts on potassium cyanide with formation of a yellowish-red precipitate of ferrous cyanide, which combines with more potassium cyanide to form ferrocyanide, $4KCy,FeCy_2$, and in presence of free acid prussian blue is formed. Ferric salts, in the absence of ferrous salts, decompose potassium cyanide with evolution of hydrocyanic acid and precipitation of ferric hydroxide in a finely-divided colloidal condition, with difficulty removed by filtration. Mixed ferrous and ferric sulphates, which are probably always present in partially oxidised pyritic tailings, cause a blue colour on cyanide for 1 of gold. (In the leaching tanks alone, 1 lb. of cyanide is generally consumed per ton of material treated.)

The gold contained in the cyanide liquor is precipitated by metallic zinc, sodium amalgam, or by electrolysis.

Zinc is used in the form of clean turnings, and appears to react on the potassium aurous cyanide according to the following equation :—$2KAuCy_2 + Zn = K_2ZnCy_4 + Au_2$. Hence theoretically 1 lb. of zinc should precipitate about 6 lbs. of gold, but in practice the actual consumption of zinc is about 1 lb. for every troy ounce of gold precipitated. This result appears to be due to the formation of a gold-zinc couple,[1] which decomposes a large quantity of cyanide electrolytically. Other cyanides of the light metals can be substituted for potassium cyanide.

Reduction of the dissolved gold by sodium amalgam instead of zinc presents many advantages, since the solutions do not become saturated with zinc compounds, and the whole of the cyanogen is restored to a condition in which it is available for dissolving gold. The amalgam may be made by direct union of sodium and mercury, but in the Molloy process is produced electrolytically.[2]

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addition of the cyanide, after the free alkali of the commercial product has been neutralised.

Before treating such pyritic ores or products with cyanide, it is necessary to subject them to a treatment with water to remove free acid and soluble salts of iron, followed by washing with a solution of caustic soda or lime to decompose the basic sulphates. Slaked lime is sometimes mixed with the tailings before commencing the cyanide treatment.

Lime is preferable to soda, as though slower in its action it decomposes the iron salts equally well, is less active in effecting hydrolysis of the cyanide, and is less energetic in attacking the zinc in the precipitation tanks.

Ferric hydroxide does not appear to be acted on by potassium cyanide, but ferrous hydroxide reacts with excess of potassium cyanide to form ferrocyanide :—$Fe(OH)_2 + 6KCy = FeCy_2,4KCy + 2KOH$.

[1] Clennell states that there is reason to believe that the black deposit formed on the zinc shavings is an actual chemical compound of gold and zinc, which forms the negative element in the electric couple.

[2] The cyanide solution passes through a shallow trough containing mercury, in which trough is an inner cylindrical vessel filled with a solution of sodium carbonate. The edges of the cylinder just dip beneath the mercury, so that the contents are entirely cut off from the outer portion of the vessel. A rod of lead dips into the soda solution and forms the anode, the lead and mercury being connected with the opposite poles of a battery, so that the sodium carbonate is electrolysed by the current. The nascent sodium combines with the mercury to form an amalgam, which at once reduces the gold in the cyanide solution to form ordinary gold amalgam and sodium cyanide, which salt is equally efficacious with potassium cyanide as a solvent of gold.

H. S. Sulman (*Jour. Soc. Chem. Ind.*, 1895, 753) has proposed the use

In the process of Siemens and Halske, the gold is deposited electrolytically, very thin lead plates being used as the cathode and iron plates as the anode. Prussian blue is formed by the reaction of the dissolved iron on the ferrocyanides produced in the leaching, and is decomposed by alkali, the solution evaporated, and the cyanide recovered by fusion with sodium carbonate. Siemens and Halske's process works well with cyanide solutions of any strength, even in presence of caustic soda, and hence renders possible the employment of very weak cyanide liquor for the extraction of the gold, thus taking advantage of the selective action and avoiding the simultaneous solution of copper.

From the waste solutions which have passed the zinc boxes, or been treated by Siemens and Halske's process, a further quantity of gold can be recovered by addition of zinc-dust, preferably freed from oxide by means of ammonia.

Ferrocyanides.

$4MCy,FeCy_2$; *i.e.*, M_4FeCy_6; or M_4Cfy^{iv}.

Ferrocyanides are present in considerable proportion in the spent oxide and ammoniacal liquor of the gas-works and also exist in the black-ash liquors of the Leblanc soda-process. Great attention has been given of recent years to the economical production of ferrocyanides, as a link in the manufacture of cyanides for gold-extraction, &c., though their direct employment for the preparation of prussian blue is a decaying industry.

The methods of recovering ferrocyanides from spent oxide and analyses of the oxide from various sources are described in a paper by J. V. Esop (*Zeits. angew. Chem.*, 1889, 305; abst. *Jour. Soc. Chem. Ind.*, viii. 881).

An ingenious process for the recovery of ferrocyanides from the ammoniacal liquor of gas-works has been devised by W. L. Rowland (*Eng. Patent*, No. 22,347, 1891). See also footuote 2, page 487.

The occurrence of ferrocyanides in the Leblanc soda-liquors

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of cyanogen bromide for renovating the cyanide solutions. It can be readily prepared by the action of bromine on potassium cyanide. The bromine can be recovered by adding hydrochloric acid to the concentrated cyanide liquors, when cyanogen bromide is re-formed.

The presence of cyanogen bromide does not interfere with the assay of cyanide liquors by the silver process. It may be determined by rendering the liquor acid with hydrochloric acid, adding a slight excess of potassium iodide solution, and titrating with standard sodium thiosulphate and starch as indicator. One c.c. of decinormal thiosulphate corresponds to 0·0053 gramme of cyanogen bromide.

has long been a hindrance to the manufacture of pure alkali by that process. Newall and Sisson (*Jour. Soc. Chem. Ind.*, 1887, p. 349) recommend a process in which the ferrocyanides obtained are used for the manufacture of prussian blue, &c.

Potassium ferrocyanide is manufactured by heating carbonate of potassium with horns, hoofs, dried blood, wool and hair clippings, feathers, leather-parings, or other animal refuse. Scrap-iron is sometimes added, but in other cases the manufacturer relies on the iron of the vessel employed, which is made of great thickness on purpose.[1] The resultant mass is lixiviated with water, and the clear liquid obtained[2] boiled down, when a first crop of crystals separates. These crystals are then purified by recrystallisation. If the potassium carbonate contain more than about three per cent. of sodium carbonate, the crystallisation is interfered with. The first crop of crystals (or "prussiate scale") contains about 97 per cent. of hydrated ferrocyanide, and on recrystallisation it is obtained with 99·8 per cent. The usual impurities are sulphate, sulphite, sulphide, thiosulphate (hyposulphite), chloride, and carbonate of potassium. Very considerable quantities of sulphate are sometimes present, which may be detected and estimated by barium chloride. In addition to these impurities, the mother-liquors contain sodium salts, thiocyanate (sulphocyanide), silicate, &c.

REACTIONS OF FERROCYANIDES.

The ferrocyanides may be regarded as compounds of ferrous cyanide with the cyanide of some other metal or basylous radical. Neither the iron nor the cyanogen is recognisable by the ordinary tests, and the ferrocyanides as a class have no marked poisonous properties.

The ferrocyanides of the light metals are soluble, but most of the ferrocyanides of the heavy metals are insoluble.

The ferrocyanides of the alkaloids have been described by H. Beckurts (*Arch. Pharm.*, ccxxviii. 347; abst. *Jour. Chem. Soc.*,

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[1] This wear of the iron pots, aggravated by the presence of sulphur in the horns, &c., used, and by the high temperature necessary for the reaction, renders the working life of the prussiate pots a very short one. The fused mass obtained, called "metal," is treated with water as described in the text; it contains cyanogen equivalent to about 16–20 per cent. of potassium ferrocyanide, which, before lixiviation, may exist as cyanide, but on treatment with water, a double decomposition occurs, thus:—

$$13KCN + Fe_2S_3 = 2K_4FeCy_6 + 2K_2S + KCNS.$$

[2] The insoluble residue from the lixiviation consists largely of carbon derived from the animal matters, and used to be a waste-product. It has recently become of value as a substitute for animal charcoal in the decolorising of paraffin-wax.

1890, 1318).[1] P. H. Walker has proposed to utilise the strychnine and dimethylaniline salts for the preparation of barium and calcium ferrocyanides (*Jour. Amer. Chem. Soc.*, 1895, p. 927).

Potassium ferrocyanide is the type of the soluble ferrocyanides. An aqueous solution of the salt gives the following reactions:—

1. On adding strong hydrochloric acid to a concentrated solution of potassium ferrocyanide, hydroferrocyanic acid is set free, and, on adding ether and shaking, white crystalline scales are deposited of a compound containing $H_4FeCy_6,2(C_2H_5)_2O$. These are readily soluble in water and alcohol. The solution is strongly acid, and absorbs oxygen from the air, with formation of Prussian blue.

2. Chlorine, bromine, hydrogen dioxide, permanganate or chromate in acid solution, and other oxidising agents convert the ferrocyanide into ferricyanide:—$2K_4FeCy_6+Br_2=2KBr+2K_3FeCy_6$.

3. When boiled with yellow mercuric oxide, oxide of iron is precipitated, while mercuric cyanide and caustic and carbonated alkali remain in solution. All ferrocyanides react similarly.

4. Silver nitrate precipitates white argentic ferrocyanide, Ag_4FeCy_6, insoluble in dilute nitric acid. The precipitate is almost insoluble in cold ammonia, but on boiling with ammonia is decomposed with formation of argentic ferricyanide and cyanide, ammonium cyanide, ferrous and ferric oxides, and metallic silver.

5. Cupric sulphate added in excess precipitates chocolate-red cupric ferrocyanide, $Cu_2''FeCy_6$. With an insufficient quantity of the reagent, brown $K_2Cu''FeCy_6$ is formed. The precipitates are insoluble in acetic or hydrochloric acid, but soluble in ammonia.

6. Zinc sulphate gives a white precipitate of zinc ferrocyanide, Zn_2FeCy_6, insoluble in hydrochloric acid. When a strongly ammoniacal solution of zinc is heated to boiling, and ferrocyanide added, a white precipitate is obtained. This reaction has been recommended by the author as a very delicate test for zinc.[2]

7. Ferrous sulphate (when quite free from ferric salt) precipitates white potassio-ferrous ferrocyanide, $K_2Fe''FeCy_6$ (Everitt's salt), which rapidly turns blue in the air.

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[1] Dunstan and Short have utilised an observation of Beckurts' for effecting the separation of strychnine from brucine (see Part ii. page 368).

[2] Hot ammoniacal solutions of zinc, so dilute as to give no reaction with ammonium sulphide or sulphuretted hydrogen, yield an immediate white turbidity with potassium ferrocyanide (*Chem. News*, xxiii. 290).

8. Ferric chloride precipitates prussian blue, ferric ferrocyanide, $Fe_4Cfy_3=Fe_7Cy_{18}=3FeCy_2,2Fe_2Cy_6$. The precipitate usually contains more or less potassio-ferric ferro-cyanide, or Williamson's blue, $KFe'''Cfy$. With an insufficient quantity of ferric salt a compound is produced, called "soluble prussian blue," which is insoluble in saline solutions but dissolves in pure water.[1] Ferric ferrocyanide is insoluble in dilute mineral acids, but dissolves in oxalic acid to a deep blue liquid (formerly used as an ink), and in ammonium tartrate to a violet liquid.

The formation of prussian blue furnishes the most delicate and characteristic test for ferrocyanides. It is also applied to the detection of simple cyanides and hydrocyanic acid (see page 429).

When boiled with caustic alkalies or magnesia, ferric ferrocyanide (prussian blue) is decomposed with precipitation of ferric hydroxide and formation of a soluble ferrocyanide.

Determination of Ferrocyanides.

The determination of ferrocyanides can be effected by precipitation, as the silver, copper, or iron salts, or by conversion into ferricyanide by oxidation with permanganate in acid solution. In applying this method, thiocyanates, sulphites, sulphides, thiosulphates, and other reducing agents must be absent. The process, which is well adapted for the assay of ferrocyanides in the absence of these impurities, is applied as follows:—A quantity of material, containing about 0·2 gramme of potassium ferrocyanide, is dissolved in water, the solution diluted to about 200 c.c., and placed in a white basin. The solution is acidified with sulphuric acid, and standard solution of potassium permanganate is run in till the yellow colour of the liquid changes to yellowish-red. The end-reaction is tolerably definite. If a trace of ferric chloride be added to the liquid, the disappearance of the bluish-green colour will render the termination still more distinct. The reaction is:—

$$5K_4FeCy_6+KMnO_4+4H_2SO_4=5K_3FeCy_6+3K_2SO_4+MnSO_4+4H_2O.$$

The permanganate is preferably set by titrating a known quantity of pure potassium ferrocyanide, but the ordinary decinormal solution may be employed. Each c.c. of decinormal permanganate used represents 0·04224 of crystallised, or 0·03684 of anhydrous, potassium ferrocyanide.

From salts of all other kinds than chlorides, bromides, iodides, iodates, cyanides, thiocyanates, ferricyanides, and sulphides, ferrocyanides may be separated by precipitating the liquid with silver nitrate in presence of free nitric acid.

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[1] Guignet suggests soluble prussian blue as very suitable for anatomical injections, since it remains in suspension in presence of a large amount of gelatin.

From chlorides and bromides (and thiocyanates) ferrocyanides may be separated by cupric sulphate in presence of free acid, and from these salts and from ferricyanides by precipitation with ferric sulphate.

In the filtrate from the copper precipitate, after removing the excess of copper by sulphuretted hydrogen, the alkaline base of soluble ferrocyanides can be conveniently determined.

Prussian blue and other insoluble ferrocyanides (the silver salt imperfectly) are converted into potassium ferrocyanide by boiling with solution of caustic alkali, the heavy metal being usually precipitated as oxide. If the metallic oxide be soluble in excess of alkali solution, it may be got rid of by passing carbon dioxide through the liquid, or in some cases magnesia may be substituted for the potash or soda. In the filtrate, the ferrocyanide can be determined by standard permanganate or other means.

The mother-liquors from ferrocyanide works may be assayed for ferrocyanide as follows:—Remove any sulphide by boiling the liquid with lead carbonate; filter, acidify the filtrate with dilute sulphuric acid, and add from a burette a standard solution of cupric sulphate, containing 10 grammes of the crystallised salt to the litre. The addition is continued until a strip of filter-paper, immersed so that the clear liquid may rise by capillary attraction, gives no blue colour when touched with a drop of ferric chloride. The precipitating power of the copper solution is ascertained by means of pure potassium ferrocyanide. About 0·2 gramme of the salt should be used, dissolved in 50 c.c. of water. Thiocyanates (sulphocyanides) do not interfere with this method.

O. Knublauch (*Jour. Soc. Chem. Ind.*, 1889, p. 733) takes a convenient quantity of the ferrocyanide solution and adds to it slightly more copper sulphate solution than is required, as shown by the ferric chloride indicator applied as above; too little copper sulphate causing decomposition even with pure ferrocyanide. The solution is next filtered, poured into hot ferric chloride solution (containing 60 grammes ferric chloride, and 200 c.c. per litre of hydrochloric acid of 1·19 specific gravity), and the whole filtered at about 80° C. The precipitate is washed somewhat with hot water, decomposed with a 10 per cent. solution of caustic potash, and the filtrate titrated with the standard cupric sulphate solution. If it be thought desirable, the filtrate from the prussian blue precipitate may be retreated in the same way, and the numbers obtained by titration added as a correction to the first result.[1]

Instead of using cupric sulphate, a solution of ferric sulphate or chloride may be employed. When this is added with vigorous agitation to a liquid containing a soluble ferrocyanide, a deep blue liquid results, which on a further addition becomes turbid, and when exactly sufficient iron solution has been added for the reaction, $3K_4Cfy + 4FeCl_3 = 12KCl + Fe_4Cfy_3$, the prussian blue coagulates, and the liquid becomes perfectly clear. The end-reaction may also be observed as in the last process. If thiocyanates be present, the least excess of iron solution will cause the liquid to assume a deep red colour. The change from blue to red furnishes a very definite end-reaction.

This process may be conveniently employed for the assay of ferrocyanide in dye-vats and in "metal," which is the name given to the crude product in the manufacture of ferrocyanide. In the case of alkaline liquids, the solution must be first acidulated with dilute sulphuric or nitric acid.

When the mother-liquors of ferrocyanide works containing 15 per cent. or less of potassium ferrocyanide are to be assayed, J. Tscherniac (*Zeits. Anal. Chem.*; abst. *Chem. News*, xlvii. 254) operates as follows:—Ten c.c. measure of the solution is poured into 70 c.c. of alcohol of 95 per cent., to which a little acetic acid has been previously added. The precipitated ferrocyanide, after washing with alcohol of 90 per cent. until the washings are colourless, is dried at 100° C. on the filter, dissolved in water, and titrated with standard permanganate solution.

The following method for the examination of ferrocyanides, and for determining the value of prussiate melt and estimating the ferrocyanide in spent "mass" from the gas purifiers has been described by R. Zaloziecki (*Zeits. Anal. Chem.*, xxx. 484; abst.

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[1] Moldenhauer and Leybold state that, when using Knublauch's method, the end of the titration was often uncertain. They recommend the following procedure:—50 grammes of the spent substance are placed in a flask with 100 c.c. of a solution of 10 per cent. caustic soda and 2 per cent. sodium carbonate, heat is applied to hasten the decomposition, and the whole then diluted to 1030 c.c. 100 c.c. of the filtrate is evaporated down in a platinum or porcelain basin and treated with 25 c.c. of dilute sulphuric acid (10 per cent.). After further evaporation the excess of acid is driven off and the organic matter destroyed by ignition. The yellow residue consists of ferric sulphate and sodium sulphate, which is dissolved in dilute sulphuric acid. The iron is reduced to the ferrous state by means of zinc and titrated with permanganate solution. From the iron thus found the amount of prussian blue is calculated. A blank experiment should be made to ascertain the amount of a slight correction for impurities in the zinc.

Chem. News, 1891, p. 207). It is based on the fact that ferrocyanide of potassium or sodium may be completely precipitated in the form of double ferrocyanides of zinc and alkali metal by the addition of zinc carbonate, and subsequent passage of a current of carbonic acid gas. The double ferrocyanide is then transformed into the corresponding sodium or potassium carbonate, and the quantity of ferrocyanide originally present found by titrating the alkaline carbonate formed. According to Zaloziecki, 3 molecules of potassium ferrocyanide yield on decomposition 2 molecules of zinc ferrocyanide, whilst 1 molecule of potassium ferrocyanide remains undecomposed. The double salt, therefore, corresponds to the formula $2Zn_2FeCy_6+K_4FeCy_6$, its decomposition by the zinc carbonate being represented by the following equation :—

$$3K_4FeCy_6+4ZnCO_3=2Zn_2FeCy_6+K_4FeCy_6+4K_2CO_3.$$

With potassium ferrocyanide the reaction takes place hot or cold. With sodium ferrocyanide the above reaction only takes place in hot solution; the reaction in the cold giving a double salt poorer in zinc. For this reason, it is necessary always to operate with hot solutions. Four parts of carbonate found, therefore, correspond to three parts of the ferrocyanide in the original substance.

A very simple method of determining small quantities of ferrocyanides present in soda-lyes has been described by F. Hurter (*Chem. News*, xxxix. 25). These liquors contain sodium ferrocyanide, cyanate, and thiocyanate, but of these the first only is objectionable, on account of the brownish colour it imparts to the finished product. Sodium cyanide may occasionally be present, in which case it may be converted into ferrocyanide by adding a small quantity of ferrous sulphate, boiling, and filtering. The following are the details of Hurter's method :—100 c.c. of the strong soda-lye are boiled with solution of bleaching powder in quantity sufficient to convert all sulphides and thiosulphates into sulphates, and the ferrocyanide into ferricyanide. The liquid is then acidified and freed, as far as possible, from the excess of chlorine by warming and agitating it. It is then titrated with $\frac{N}{20}$ solution of cupric nitrate, prepared by dissolving 3·170 grammes of metallic copper in as little nitric acid as possible, and diluting to 1 litre. On adding this solution to the acidulated liquid containing ferricyanide, a yellow precipitate of cupric ferricyanide is formed. Drops of the thoroughly-mixed liquid are taken up with a glass rod, and added to drops of a 1 per cent. solution of crystallised ferrous sulphate on a porcelain plate. As long as insufficient copper solution has been added to combine with the whole of the ferricyanide present, the deep blue ferrous ferricyanide is formed on the porcelain. When the liquid no longer contains soluble ferricyanide, the indicator acts on the copper precipitate, and reduces it to the characteristic chocolate-coloured cupric ferrocyanide. Hence, the end of the reaction is indicated by a brown colour being produced on the porcelain instead of the blue first obtained. Each c.c. of the copper solution added before this result is obtained represents ·01013 gramme of sodium ferrocyanide in the liquid. The method is not suitable for the determination of large quantities of ferrocyanides, as the colour of the copper precipitate obscures the blue colour, and the precipitate is not always of definite composition.

G. Lunge, in comparing this method with the permanganate method described on p. 465, used (*Jour. Soc. Chem. Ind.*, 1882, 91) a modification suggested by Schäppi, which consisted in avoiding the excess of bleaching powder solution used for oxidation purposes; he finds, however, that the modification is unsuitable when soda-liquors are under examination, and therefore recommends Hurter's original process as described above.

It is evident that this process is also adapted for the direct estimation of small quantities of ferricyanide, into which the ferrocyanide has first to be converted.

K. Zulkowsky (abst. *Jour. Chem. Soc.*, 1884, 501) recommends a process for the assay of ferrocyanide melt which is based on the reaction which occurs between potassium ferrocyanide and a soluble zinc salt. A standard solution of zinc sulphate is acidified with sulphuric acid, heated to boiling, and the ferrocyanide solution next run in from a burette. The point when ferric chloride gives a blue coloration to a strip of filter-paper moistened with a drop of the mixed solutions shows the end-reaction. Care must be taken that none of the zinc precipitate touches the ferric chloride on the filter-paper.

In applying the above process, R. Gasch (abst. *Jour. Chem. Soc.*, 1890, 834) employs a 1 per cent. solution of uranium acetate, with which indicator ferrocyanides give a brown coloration. Gasch also uses a standard 2 per cent. solution of potassium ferrocyanide, against which he titrates the zinc solution, instead of using a standard solution of zinc sulphate as in the original process of Zulkowsky. For the determination of ferrocyanides in old gas waste, &c., Gasch rubs 20 grammes of the substance in a mortar with 15 to 20 per cent. of caustic soda, when warm water is added until the solution is of a thin consistency. It is next made up to a known volume, filtered, and poured into a burette, and titrated as above, using uranium acetate solution as indicator. When the ferrocyanide is only present in very small amount, it is preferably precipitated as prussian blue, filtered, and dissolved in caustic alkali solution, when it is titrated as in the preceding case.

POTASSIUM FERROCYANIDE. K_4FeCy_6, *i.e.*, $4KCy,FeCy_2$; or K_4Cfy.

This important salt, known in commerce as "yellow prussiate of potash,"[1] crystallises in amber-yellow, deeply truncated octahedra of the quadratic system,[2] often having the appearance of tables. The crystals have a very perfect cleavage at right angles to the principal axis. In a crystalline state potassium ferrocyanide contains $3H_2O$, the whole of which is expelled at 100° C. At a red heat the salt is decomposed into potassium cyanide, iron carbide, and nitrogen gas.

Potassium ferrocyanide is tolerably stable at ordinary temperatures, both in the solid state and in solution. The salt is soluble in four parts of cold or in two of boiling water; but is insoluble in alcohol. It has a perfectly neutral reaction to litmus, methyl-orange, and phenolphthalein. It is not poisonous (Carles, *Jour. Chem. Soc.*, lviii. 281). Dilute acids liberate hydroferrocyanic acid from potassium ferrocyanide. By moderately dilute hot sulphuric acid the salt is decomposed with evolution of hydrocyanic acid (see page 443). When heated with excess of strong sulphuric acid, carbon monoxide is evolved as gas, and potassium, ammonium and iron sulphates are formed:—

$$K_4FeC_6N_6 + 6H_2SO_4 + 6H_2O = 6CO + 2K_2SO_4 + 3(NH_4)_2SO_4 + FeSO_4.$$

The manufacture of potassium ferrocyanide is described on page 463, and its analytical reactions on page 464.

The physiological action of potassium ferrocyanide on animals has been investigated by Combemale and Dubiquet (abst. *Jour. Chem. Soc.*, 1891, p. 99). Their results show that this salt is not poisonous, even when given to animals in doses of 2 grammes per kilogramme of body-weight. In those animals which do not vomit, a diuretic action is observed three hours after the administration even in small doses. Repeated doses of the salt cause intestinal troubles in the dog, and vomiting ensues if the dose given exceeds 80 centigrammes per kilogramme of body-weight. It is pointed out by the above-named observers that potassium ferrocyanide, in its passage through the body, is transformed into the ferricyanide, and as such is eliminated in the urine. They suggest that its diuretic action may be due to this transformation.

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[1] German:—*Blutlaugensalz*. French:—*Prussiate jaune de potasse*.

[2] The crystalline form of potassium ferrocyanide is, according to recent researches, more probably monoclinic.

SODIUM FERROCYANIDE, Na_4FeCy_6, crystallises in transparent, yellow, oblique rhombic prisms, containing, according to L. Pebal, ten molecules of water of crystallisation (*Jour. Chem. Soc.*, 1860). The crystals effloresce and fall to powder on exposure to warm air. The salt is soluble in 4½ parts of cold water, but is insoluble in alcohol. Sodium ferrocyanide is manufactured on a considerable scale in Philadelphia.

PRUSSIAN BLUE.

Prussian blue is commonly described as ferric ferrocyanide, a compound which contains Fe_7Cy_{18}; *i.e.*, $3FeCy_2,4FeCy_3$. The commercial product, however, is, or may be, a mixture of true Prussian blue with Turnbull's blue, Williamson's blue, and possibly other cyanides of iron. The following formulæ show the relationship of these and allied compounds (Cfy being FeC_6N_6):—

Fe_3Cy_6	Ferrous Ferrocyanide.	$Fe_2''Cfy^{iv}$
$K_2Fe_2Cy_6$	Potassio-ferrous Ferrocyanide. Everitt's salt.	$K_2Fe''Cfy^{iv}$
KFe_2Cy_6	Potassio-ferric Ferrocyanide.[1] Williamson's blue.	$KFe'''Cfy^{iv}$
$K_2Fe_4Cy_{12}$	Di-potassio-diferric Diferrocyanide. Soluble Prussian blue.	$K_2Fe_2'''Cfy_2^{iv}$
Fe_7Cy_{18}	Ferric Ferrocyanide. Prussian blue.	$Fe_4'''Cfy_3^{iv}$
KFe_2Cy_6	Potassio-ferrous Ferricyanide.[1] Williamson's blue.	$KFe''Cfy'''$
Fe_5Cy_{12}	Ferrous Ferricyanide. Turnbull's or Gmelin's blue.	$Fe_3''Cfy_2'''$

Prussian blue[2] is obtained in the manner and has the properties described on page 465. When dry it forms a dark blue amorphous substance, having a strong coppery lustre and conchoidal

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[1] Which of the formulæ given for Williamson's blue is correct is uncertain, and the problem is apparently beyond solution. $KFe'''Cfy^{iv}$ and $KFe''Cfy'''$ have the same ultimate composition, and yield the same products on treatment with alkalies. Soluble Prussian blue appears to be isomeric with Williamson's blue.

[2] Prussian blue is manufactured in a great variety of shades. The lighter are useful for the manufacture of zinc-greens, whilst the darker are employed for the preparation of chrome-greens. According to J. C. Gentele, the best blues are obtained by treating, in the first instance, a ferrous salt with yellow prussiate, and then oxidising the resultant bluish-white precipitate of ferrous ferrocyanide. This may be effected by nitric acid, bleaching powder, and hydrochloric acid, &c. In the process of Crowther and Rossiter (*English Patent*, No. 17,846, 1893), ferrous ferrocyanide, or Everitt's salt, is suspended in strongly acidulated water and submitted to electrolysis. A blue of extremely vivid violet reflex is obtained at the anode. The hydrogen evolved at the cathode can be taken up by manganese dioxide or organic nitro-derivatives.

The pigment which in German commerce goes by the name of "Prussian fracture. The characteristics of good prussian blue are lightness; and a deep, fine, blue colour. A coppery lustre is usual, but not an essential character. Prussian blue should adhere strongly to the tongue. It should not effervesce with acids, nor thicken when boiled in water.

Prussian blue is often adulterated with alumina, starch, barium sulphate, calcium carbonate, &c. The last may be detected by the effervescence on addition of dilute hydrochloric acid. In the solution obtained by digestion with the acid for some time, alumina may be detected by addition of ammonia. Starch may be detected by boiling the sample with water, which will produce paste in presence of a large proportion. Smaller quantities may be detected by digesting the finely-powdered sample with magnesia and water in the cold; the residue is filtered and washed, and treated with cold dilute hydrochloric acid. The oxide of iron and excess of magnesia are dissolved, and the residual starch can be weighed, examined under the microscope, and tested with iodine. Any china-clay and barium sulphate will remain with the starch, and, after removing the latter by ignition or boiling with water, the residue may be examined with a view to their recognition.

The proportion of real ferrocyanide contained in prussian blue may be determined by treating the sample with caustic alkali, filtering from the iron oxide, and determining the ferrocyanide in the filtrate as described on page 465 *et seq.*[1]

The colouring power of prussian blue may be tested by grinding the sample with a large proportion of white-lead and oil, and comparing the colour with that given by a standard sample of known purity.

Ferricyanides.

$3MCy,FeCy_3$; *i.e.*, $M_3(FeCy_6)'''$; M_3Cfy'''; or M_3Cfdy'''.

The ferricyanides may be regarded as compounds of ferric cyanide, $FeCy_3$, with the cyanide of some other metal or basylous radical. They are obtained by the action of chlorine or other oxidising (*i.e.*, dehydrogenating) agents on the ferrocyanides:—

$$2M_4FeCy_6 + Cl_2 = 2MCl + 2M_3FeCy_6.$$

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blue" is "Paris blue" (*i.e.*, the best quality of ferrocyanogen-blue) mixed with starch, barium sulphate, gypsum, burned and finely ground kaolin, or other diluents. Very low varieties of blue are often "faced" by making the dried blue rotate in a cask charged with fine dust of pure Paris blue.

[1] Parry and Coste (*Analyst*, 1896) find that if the nitrogen of commercial prussian blue (as determined by Kjeldahl's process) be multiplied by 4·4, or the total iron by 3·03, the product represents very closely the amount of pigment in the sample.

Potassium ferricyanide is manufactured in practice by cting on potassium ferrocyanide, either as coarse powder or in lution, by chlorine gas. The product is separated from the otassium chloride by crystallisation. The mother-liquor is mployed for preparing Turnbull's blue (page 477) by precipi- tion with ferrous sulphate. The crude mixture of potassium rricyanide and chloride is sometimes employed direct by lico-printers.

The above process of preparing potassium ferricyanide is unsatis- ctory as a laboratory method, since iron ferrocyanides ("Prussian reen"[1]) are simultaneously formed.

M. S. **Walker** (*Amer. Chem. Jour.*, xvii., 1895, p. 68) prefers use potassium permanganate as the oxidising agent.[2]

In the laboratory, potassium ferricyanide is readily prepared by eating potassium ferrocyanide with an equivalent quantity of romine.[3]

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[1] *Prussian green* may be prepared by saturating a solution of potassium rricyanide with chlorine, excluding the light, and heating the liquid to iling. The product is allowed to cool in a current of chlorine, and the cess of chlorine washed out with cold water.

A. M. **Clark** (*English Patent*, No. 22,558, 1891) treats a mixture of tassium and calcium ferrocyanides with potassium permanganate, when e following reaction is stated to occur:—

$$3Ca_2Cfy^{iv} + 7K_4Cfy^{iv} + 2KMnO_4 = 10K_3Cfy''' + 2MnO + 6CaO.$$

The manganous oxide and the greater part of the lime remain undis- lved, and the small quantity of the latter base which is contained in the tered liquid may be precipitated by carbonic acid. An equally pure product said to be obtainable by electrolysis.

[2] Walker operates as follows:—Twenty-six parts of potassium ferrocyanide re dissolved in 200 c.c. of cold water, and 8 parts of strong hydrochloric cid added. Two parts of potassium permanganate in 300 parts of water are hen introduced slowly. The solution should give a brown colour, but no recipitate with ferric chloride solution. The excess of acid is neutralised ith calcium or barium carbonate, and the solution evaporated on the water- ath. The first crystals will be pure, whilst the subsequent crops of rystals may contain chlorides, which can be eliminated by fractional rystallisation.

[3] The action of bromine on potassium ferricyanide when heated in sealed bes to 100°–120° C. is somewhat complex, and has been investigated by . J. **Reynolds** (*Jour. Chem. Soc.*, liii. 767).

K. **Seuberlich** (abst. *Jour. Chem. Soc.*, 1881, p. 239) has pointed out e conditions of success in the preparation of potassium ferricyanide from rrocyanide and lead peroxide. Since free alkali is formed in the reaction, it ust be neutralised by the addition of an acid. A solution of potassium rrocyanide is treated in the cold with the required quantity of dilute ydrochloric acid and an excess of lead peroxide. The ferricyanide is

By the action of iodine on its hot aqueous solution, potassium ferricyanide is converted into a new salt, *potassium perferricyanide*, of the formula K_2FeCy_6.

Neither the iron nor the cyanogen of ferricyanides can be recognised by the ordinary tests, and the salts as a class are not violently poisonous. The ferricyanides of the light metals have a red colour, and are soluble in water. The ferricyanides of the heavy metals are mostly insoluble.

By the action of heat and strong acids, the ferricyanides are decomposed in a very similar manner to the ferrocyanides (p. 443).

An aqueous solution of potassium ferricyanide gives the following reactions:—

1. Sulphuretted hydrogen readily reduces ferricyanide with formation of a ferrocyanide.

2. In presence of caustic alkali, stannous, manganous, ferrous, plumbous, and chromic oxides reduce ferricyanides to ferrocyanides. Alcohol, oxalates, cyanides, sulphites, and phosphites also exert a reducing action, and indigo is bleached. Sugar, starch, and cellulose likewise reduce ferricyanide. Prud'homme (abst. *Jour. Chem. Soc.*, 1891, page 410) has described a number of cases in which these and similar reactions are reversed.

3. Hydrogen peroxide is decomposed by alkaline ferricyanide, with evolution of oxygen (see page 476).

4. When boiled with yellow mercuric oxide and water, ferri-

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obtained from the filtered liquid by concentration, when it crystallises out almost pure. Schonbein's method (abst. *Jour. Chem. Soc.*, 1881, p. 323) consists in passing a stream of carbon dioxide through the boiling mixture of ferrocyanide and lead peroxide.

A similar process for the manufacture of potassium ferricyanide has been described by G. Kassner (*Chem. Zeit.*, xiii. 1701; abst. *Jour. Soc. Chem. Ind.*, ix. p. 391). It consists in adding calcium plumbate to a solution of potassium ferrocyanide and passing a stream of carbonic acid gas when the reaction proceeds according to the equation:—

$$2K_4FeCy_6 + PbO_2(CaO)_2 + 4CO_2 = 2K_3FeCy_6 + K_2CO_3 + PbCO_3 + 2CaCO_3.$$

The carbonates of lead and calcium separate as an insoluble precipitate which can be filtered off and regenerated by a simple roasting, whilst the solution contains potassium ferricyanide in a pure form and potassium carbonate as a valuable by-product. The calcium plumbate is prepared by roasting oxide or carbonate of lead with calcium carbonate at a low red heat. In the conversion of potassium ferrocyanide into ferricyanide by means of lead peroxide a quantity of potassium hydrate is set free which must be neutralised in some way before the reaction can go on to completion, and carbonic acid suffices for the neutralisation.

cyanides are completely decomposed with formation of mercuric cyanide and precipitation of oxide of iron.

5. Silver nitrate precipitates orange-red argentic ferricyanide, Ag_3FeCy_6, insoluble in dilute nitric acid, but soluble in ammonia.

6. Ferrous sulphate produces a deep blue precipitate (Turnbull's blue) of ferrous ferricyanide, $Fe_3''(FeCy_6)_2$, insoluble in dilute acids, but decomposed by hot caustic alkali with formation of soluble ferrocyanide and black ferroso-ferric oxide. This reaction distinguishes it from prussian blue, which in appearance it closely resembles, but which yields yellow-brown ferric hydroxide, without any ferrous oxide, on boiling with alkalies.

7. Ferric chloride, if free from ferrous salt, produces merely a brownish coloration in solutions of ferricyanides free from ferrocyanides. The resultant liquid is a very delicate test for reducing bodies which cause the formation of a blue precipitate (see Ptomaïnes, page 328).

8. When boiled with potassium cyanide, a solution of potassium ferricyanide is reduced to ferrocyanide, whilst free hydrocyanic acid, ammonia, and carbon dioxide are simultaneously formed.

9. When treated with a nitrite and acetic acid, ferricyanides are converted into nitroprussides (see page 477).

DETERMINATION OF FERRICYANIDES.

Ferricyanides may be determined by boiling with ferrous sulphate and caustic alkali, filtering, and determining the ferrocyanide in the filtrate by permanganate (see page 465). Sodium sulphite or thiosulphate may be substituted for the ferrous sulphate, if the process with a ferric solution described on page 467 be used instead of titration with permanganate. In either case, any pre-existing ferrocyanide must be determined in a separate portion, and the quantity subtracted from the total amount found.

Another method is to mix the dilute solution of the ferricyanide with potassium iodide and hydrochloric acid in excess, add an excess of solution of iron-free zinc sulphate, neutralise the free acid with a slight excess of sodium bicarbonate, and determine the liberated iodine by standard sodium thiosulphate (hyposulphite) and starch. The reaction is as follows:—

$$2K_3FeCy_6 + 4ZnSO_4 + 2KI + 2Na_2S_2O_3 = 4K_2SO_4 + 2Zn_2FeCy_6 + 2NaI + Na_2S_4O_6.$$

Each c.c. of decinormal thiosulphate required to react on the liberated iodine represents 0·0329 gramme of potassium ferricyanide.

A very simple and fairly accurate method of determining small quantities of ferricyanides is described on page 469.

J. Quincke (*Zeits. anal. Chem.*, xxxi. 1; abst. *Jour. Chem.*

Soc., 1892, p. 527) has shown that the action of caustic alkali and hydrogen peroxide on potassium ferricyanide proceeds quantitatively according to the equation :—

$$2K_3FeCy_6 + 2KHO + H_2O_2 = 2K_4FeCy_6 + 2H_2O + O_2.$$[1]

The volume of oxygen evolved is therefore a measure of the ferricyanide present.

The best mode of operating is to introduce 5 or 10 c.c. of the solution of the ferricyanide into the closed tube of a nitrometer filled with mercury, and then run in an equal measure of caustic soda solution through the tap. This is followed by a solution of hydrogen peroxide, and the contents of the nitrometer are mixed by agitation. More hydrogen peroxide is then added to ensure the completion of the reaction, when a measure of water, equal to the combined aqueous liquids, is poured into the open limb, the level of the mercury in the two limbs adjusted, and the volume of gas read off. 1 c.c. of oxygen at 0° C. and 760 mm. = 0·02945 gramme potassium ferricyanide, or ·0279 gramme at the ordinary temperature and pressure.

Barium peroxide dissolved in hydrochloric acid may be substituted for the hydrogen peroxide, provided that an excess of strongly alkaline ferricyanide be employed.

POTASSIUM FERRICYANIDE, $K_3FeC_6N_6 = 3KCy,FeCy_3 = K_3FeCy_6$.—This salt, known in commerce as "red prussiate of potash,"[2] crystallises in ruby-red monoclinic prisms, which are anhydrous. Potassium ferricyanide has an aperient action, but is not poisonous. It is soluble in 2½ parts of cold, or 1¼ of boiling water. The solution has a strong yellow colour. By exposure to light, or evaporation to dryness, potassium ferricyanide is partially decomposed with formation of ferrocyanide. The change is hastened by the presence of potassium oxalate, mercuric chloride, &c. Ammonium ferricyanide is reduced by light more readily than the potassium salt. Paper wetted with mixed solutions of a ferricyanide and ferric chloride becomes blue on exposure to light, a fact utilised in photography to obtain the so-called "ferrotype" prints.

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[1] G. Kassner (abst. *Jour. Chem. Soc.*, 1890, p. 834) proposes to determine the ferrocyanide formed in the above reaction, and so obtain an estimate of the amount of ferricyanide originally present. After acidulating the cooled liquid with dilute sulphuric acid, and boiling off the excess of hydrogen peroxide, it is titrated with standard permanganate solution. Kassner also recommends the reaction as a convenient means of preparing pure oxygen.

[2] German : — *Ferridcyankalium*, or *Rothesblutlaugensalz*. French :— *Prussiate rouge de potasse*.

Ferrous Ferricyanide, or Turnbull's Blue, Fe_5Cy_{12}; or, $Fe_3''(FeCy_6)_2'''$.—Turnbull's blue resembles prussian blue, but its colour is lighter, and is almost free from the coppery lustre of the latter pigment. Some of its properties have been described on page 475.

The composition and constitution of Turnbull's blue, as compared with that of Prussian blue, have been investigated by J. E. Reynolds (*Jour. Chem. Soc.*, li. 644), whose results showed that the two blues were not identical, being represented respectively by Williamson's formulæ Fe_5Cy_{12} and Fe_7Cy_{18}. An estimation of the water in well-dried samples gave results approximating to the composition Fe_5Cy_{12}, $12H_2O$, and Fe_7Cy_{18}, $14H_2O$.

J. Messner (abst. *Jour. Chem. Soc.*, 1895, page 486) considers that, since the percentage composition of the two blues is so similar, the result of quantitative analysis is inconclusive.[1]

Nitroso-ferricyanides.[2] Nitroprussides.

$M_2FeCy_5(NO)$.

When a ferrocyanide is heated with nitric acid, or when a ferricyanide is treated with nitrous acid, a substitution-product is formed in which one-sixth of the cyanogen is replaced by nitrosyl. The nitrous acid may be conveniently substituted by a mixture of a nitrite with acetic acid, and the addition of mercuric chloride is advantageous as a means of converting the cyanogen into a stable compound.[3] The reaction, which is very complex, is generally represented as follows:—

$$2K_3FeCy_6 + 4NaNO_2 + 2H\bar{A} + HgCl_2 = 2Na_2FeCy_5(NO) + HgCy_2 + 2KCl + 2K\bar{A} + KNO_2 + KNO_3 + H_2O.$$

Potassium nitroprusside may also be obtained by adding potassium cyanide to ferrous sulphate solution, and heating the brown precipitate with potassium nitrite:—

a. $5KCy + 2FeSO_4 = KFe_2''Cy_5 + 2K_2SO_4$; and,
b. $2KFe_2''Cy_5 + 2KNO_2 = 2K_2FeCy_5(NO) + 2FeO$.

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[1] Messner erroneously states that both Turnbull's blue and Prussian blue yield a ferrocyanide and ferric hydroxide by the action of alkalies; but as a fact the former compound yields black ferroso-ferric oxide (hydrated) and the latter brown ferric oxide, the reactions being respectively:—

For Turnbull's blue, $Fe_3''Cfy_2''' + 8K(OH) = 2K_4Cfy^{iv} + 2Fe(OH)_3 + Fe(OH)_2$.
For prussian blue, $Fe_4'''Cfy_3^{iv} + 12K(OH) = 3K_4Cfy^{iv} + 4Fe(OH)_3$.

[2] K. A. Hofmann has described nitroso-ferrocyanides (abst. *Jour. Chem. Soc.*, 1896, i. 269.

[3] See also Prud'homme, *Compt. rend.*, cxi. 45; abst. *Jour. Chem. Soc.*, 1890, p. 1387, and 1891, p. 410.

Sodium Nitroprusside, $Na_2FeCy_5NO + 2$ aqua, crystallises more readily than the potassium salt. It forms deep red crystals very soluble in water, and is decomposed on exposure to light, with formation of a blue precipitate.[1]

Soluble nitroprussides are unchanged by ferric salts. With silver nitrate they yield a flesh-coloured precipitate, insoluble in nitric acid, and salmon-coloured precipitates with ferrous and zinc salts. The last reaction distinguishes nitroprussides from cyanides of the formula M_2FeCy_6 ("perferricyanides"), which give a green precipitate with zinc sulphate.

Sulphuretted hydrogen decomposes nitroprussides with formation of a ferrocyanide, separation of prussian blue and sulphur, and production of iron nitrosulphide.

The chief interest attaching to the nitroprussides is the beautiful but transient violet colour which they yield with soluble sulphides.[2] This reaction affords a delicate test both for sulphides and for nitroprussides. If one inch of hair be fused with sodium carbonate and the product dissolved in water, the presence of sulphur is recognisable by subsequently adding a nitroprusside.

Sodium nitroprusside is also applied as a test for creatinine (page 294), which in presence of caustic alkali gives a fine ruby-red coloration, changing in a few minutes to a straw-yellow. Acetone also gives a red colour with alkaline nitroprusside solution, and other ketones and aldehydes give reactions ranging from yellowish-red to violet (B. von Bittó, *Jour. Soc. Chem. Ind.*, 1892, p. 847).

When a solution of a nitroprusside is rendered alkaline by caustic soda it acquires a red tint, changing to orange, and on boiling the liquid the nitrosyl group exerts a reducing action, ferrous hydroxide being precipitated, nitrogen evolved, and sodium ferrocyanide and nitrite formed.

Cobalticyanides. $3MCy,CoCy_3$; or M_3CoCy_6.

This very permanent class of double cyanides is chiefly of interest from its application to the separation of nickel and cobalt. When potassium cyanide is added to a solution of cobalt, brownish-white cobaltous cyanide, $CoCy_2$, is formed; this dissolves in excess of potassium cyanide to form the easily decomposable double cyanide, $2KCy,CoCy_2$. With excess of potassium cyanide, red potassium cobalto-cyanide, $4KCy,CoCy_2$, is formed. On heating the solution containing this, it is quickly converted, with evolution of hydrogen, into colourless very stable cobalticyanide of potassium, K_3CoCy_6, analogous to the ferricyanide. A more perfect reaction occurs on treating the cold solution with chlorine or bromine. The resultant solution of potassium cobalticyanide is not decomposed by boiling with mercuric oxide. This reaction enables nickel to be separated from cobalt, the former of which is wholly precipitated, and remains as NiO on igniting the precipitate. The same reaction distinguishes cobalticyanides from ferrocyanides and ferricyanides. Cobalticyanides give no precipitate with ferric salts. They are completely precipitated from acid solutions by nickel sulphate, the precipitate leaving Ni_3+Co_2 by ignition in the air and subsequent reduction in hydrogen. Mercurous nitrate completely precipitates cobalticyanides from neutral solutions, the precipitate leaving Co_3O_4 on ignition. Cobalticyanides are completely decomposed by heating with concentrated sulphuric acid (page 441). By treatment with fuming nitric acid, potassium cobalticyanide yields a red substance stated to have the composition, $KH_2Co_3Cy_{11}+H_2O$ (Jackson and Comey, *Ber.*, xxix. 1020).

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[1] Sodium nitroprusside was found by Eder to be twenty times more sensitive to light than potassium ferricyanide, and in presence of ferric chloride the decomposition was still more rapid.

[2] By operating in alcoholic solution the colouring matter separates as a purple-blue oily compound, which gives a green powder when dried *in vacuo*. It readily decomposes into a sulphide and ferricyanide.

Platinocyanides. $2MCy,PtCy_2$; or M_2PtCy_4.

The platinocyanides are of interest for their remarkable fluorescent properties, which cause them to appear strongly dichroïc. They become visible when subjected to the Röntgen dark rays, and a surface painted with barium platinocyanide and exposed while still moist to these rays, glows wherever it has not been protected by a metallic or other screen impervious to the rays. In this manner the effects of Röntgen rays may be rendered visible without resource to photography.

The platinocyanides of the light metals are soluble in water and crystallise well. Those of the heavy metals are mostly insoluble and can be prepared by precipitation. The platinocyanides are extremely stable, even boiling sulphuric acid decomposing them but slowly. When treated in solution with nitric acid, chlorine, or bromine, the platinocyanides form addition-compounds. Thus on passing chlorine into a hot solution of potassium platinocyanide, the compound $K_2PtCy_4Cl_2+2$ aqua is deposited in colourless crystals on evaporation. On treating these with a strong solution of the platinocyanide, they are converted into copper-red needles which are said to have the composition—

$$5K_2PtCy_4, K_2PtCy_4Cl_2, 3H_2O+18 \text{ aqua}.$$

This compound, when boiled with caustic potash, yields potassium platinocyanide, chloride, and hypochlorite. Unlike the ferrocyanides and ferricyanides, the platinocyanides are not decomposed by digestion with mercuric oxide. Mercuric chloride throws down white mercuric platinocyanide, $HgPtCy_4$. Mercurous nitrate in small quantity yields a white precipitate, but when added in excess, a highly characteristic bright blue precipitate is obtained. Cupric sulphate yields a flocculent blue or green precipitate containing $CuPtCy_4$, which, when suspended in water and decomposed by sulphuretted hydrogen, yields free hydroplatinocyanic acid. On evaporating the filtrate to dryness, and recrystallising the residue from a mixture of alcohol and ether, the acid is obtained in bluish-black hydrated prisms, or greenish-yellow needles, having a coppery or golden lustre, which turn yellow and deliquesce on exposure to air.

Potassium Platinocyanide, $2KCy,PtCy_2 + 3H_2O$, is obtained by dissolving ammonium chloroplatinate and a little caustic potash in a concentrated boiling solution of potassium cyanide, and recrystallising the product from water.

A. Schertel (*Ber.*, xxix. 204) prepares the salt by dissolving well-washed and recently-precipitated platinum sulphide in a solution of potassium cyanide, and concentrating the resultant colourless liquid. If commercial potassium cyanide containing much sodium cyanide be employed, crystals of the composition $KNaPtCy_4 + 3H_2O$ are obtained, and from the mother-liquor *sodium platinocyanide*, $Na_2PtCy_4 + 3H_2O$, crystallises out in long, transparent, colourless needles, permanent in the air, and *not* fluorescent. The salt may also be conveniently prepared by precipitating a solution of potassium platinocyanide with copper sulphate, and treating the washed precipitate with a slight excess of caustic soda.

Potassium platinocyanide forms yellow rhombic crystals exhibiting a blue dichroïsm. On exposure to air it effloresces and becomes nearly white. The salt is readily soluble in hot water, but is in great part deposited on cooling. Cold concentrated sulphuric acid decomposes it with formation of platinous cyanide.

Barium Platinocyanide, $BaCy_2,PtCy_2 + 4$ aqua, may be prepared in a manner similar to the potassium salt; or hydrocyanic acid vapours may be passed into a solution of platinic chloride holding barium carbonate in suspension, until oxygen and carbon dioxide cease to be evolved :—

$$PtCl_4 + 3BaCO_3 + 4HCy = BaPtCy_4 + 2BaCl_2 + 2H_2O + 3CO_2 + O.$$

Barium platinocyanide forms monoclinic prisms which appear green in the direction of the principal axis, but sulphur-yellow with a blue-violet sheen in a direction at right angles to the axis. The salt dissolves in about thirty-three parts of cold water, but is considerably more soluble at the boiling point.

MAGNESIUM PLATINOCYANIDE, $MgCy_2,PtCy_2 + 7$ aqua, is obtained by precipitating the barium salt with magnesium sulphate. It crystallises in large prisms, which are powerfully fluorescent and dichroïc. When crystallised from alcohol or from water at 70°, a yellow salt containing 6 aqua is obtained, which at 100° is converted into a white hydrate containing 2 aqua, and this at 180° gives a yellow anhydrous salt.

CYANATES.

A series of compounds having the composition of cyanates or oxycyanides are produced by the action of oxidising agents on cyanides. The metallic cyanates are obtainable by the following reactions:—

1. By passing cyanogen gas into the solution of the hydroxide of an alkali-metal or alkaline earth metal:—

$$2KHO + Cy_2 = KCyO + KCy.$$

2. By heating a carbonate of alkali-metal to low redness with mercuric cyanide.

3. By the electrolysis of a solution of the corresponding cyanide, the cyanate being formed at the anode.

4. By fusing a cyanide or ferrocyanide with an oxidising agent, such as manganese dioxide, red lead, litharge, potassium bichromate, nitre, &c.

5. By the action of a highly alkaline solution of a hypochlorite on urea (see page 276).

6. By the action of a hypobromite on a cyanide (A. H. Allen).

7. By the action of an alkaline solution of permanganate on a cyanide.

The alkyl cyanates are compounds of great theoretical interest, but they have received no practical application.

Hydrogen Cyanate. Cyanic Acid.

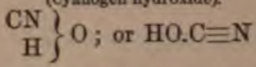
This compound cannot be prepared by the action of mineral acids on metallic cyanates, since, at the moment of its formation, the greater part is decomposed into carbon dioxide and ammonia, the latter compound remaining in combination with the mineral acid used:— $KCNO + H_2O + 2HCl = KCl + NH_3,HCl + CO_2$. Sufficient cyanic acid escapes decomposition to give to the evolved gas a pungent odour which excites tears.

Cyanic acid may, however, be obtained by heating its polymer, anhydrous cyanuric acid, nearly to redness in a current of carbon dioxide. A mixture of urea with phosphoric anhydride, or of uric acid with sulphuric acid and manganese dioxide, may be substituted for the cyanuric acid.

Cyanic acid is a colourless liquid, having an extremely pungent odour resembling that of sulphurous or glacial acetic acid. It is extremely unstable, becoming rapidly polymerised into a snow-white, insoluble substance called cyamelide, $(CNHO)_n$.

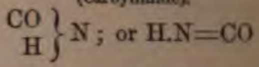
Two isomeric forms of cyanic acid have a possible existence, represented respectively by the following formulæ:—

NORMAL CYANIC ACID
(Cyanogen hydroxide).



$$\left.\begin{matrix}CN\\H\end{matrix}\right\}O;\ \text{or}\ HO.C{\equiv}N$$

ISO-CYANIC ACID
(Carbylimide).



$$\left.\begin{matrix}CO\\H\end{matrix}\right\}N;\ \text{or}\ H.N{=}CO$$

Only one modification of cyanic acid is known in the free state, and the constitution of this is not certain, some authorities regarding it as the *normal acid* and others as *iso*cyanic acid. The same difference of opinion exists as to the constitution of the ordinary metallic cyanates (see below). The *alkyl normal cyanates* are almost unknown, as they polymerise with great facility into the corresponding cyanurates. The *alkyl isocyanates* are obtainable as volatile pungent liquids, readily polymerising to isocyanurates.

Metallic Cyanates.

As stated above, the molecular constitution of the metallic cyanates is uncertain. Some authorities, arguing from the fact that potassium cyanate, when distilled with potassium ethyl sulphate, yields ethyl isocyanate, regard the ordinary potassium salt as an *iso*cyanate. Other chemists think too much stress is laid on this and allied facts, since frequent observations have shown that normal cyanic compounds readily isomerise. Similarly, allyl thiocyanate readily changes into the isothiocyanate, and the normal cyanuric esters change to the corresponding isocyanuric esters.[1]

POTASSIUM CYANATE, KO.CN; or KCyO.

This salt may possibly be the isocyanate, K.N:C:O.[2] It may be obtained by the reactions given on page 481. The best practical method of preparing it consists in fusing potassium cyanide with litharge or manganese dioxide, or potassium ferrocyanide with potassium bichromate[1] (compare page 249). Another convenient method of obtaining potassium cyanate is the oxidation of a cold solution of potassium cyanide by permanganate in presence of caustic potash (J. Volhard, abst. *Jour. Chem. Soc.*, 1891, page 160).

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[1] It is conceivable that the mother-substances may possess two constitutional formulæ, *i.e.*, that they are "tautomeric," and by the wandering of an atom of hydrogen their atoms may sometimes arrange themselves in one, and sometimes in the other form, and that they may accordingly show the reactions of either.

[2] R. Otto (*Ber.*, xxvii. 837) has obtained an isomer of ordinary potassium cyanate in broad tablets or small pyramidal crystals having a vitreous lustre. It is isomorphous with thallium thiocyanate.

Potassium cyanate crystallises in colourless scales, fusible below a red heat to a colourless liquid. It is readily soluble in water, and tolerably soluble in boiling rectified spirit, but is insoluble in absolute alcohol.

Potassium cyanate is not decomposed by exposure to or even by ignition in dry air, but in presence of moisture or by evaporation of its aqueous solution it suffers hydrolysis according to the following equation:—$2KOCN + 3H_2O = K_2CO_3 + 2NH_3 + CO_2$. This reaction is the cause of the ammoniacal smell of deliquesced commercial potassium cyanide, which often contains much cyanate. On addition of moderately concentrated sulphuric or hydrochloric acid to potassium cyanate, the greater part of the cyanic acid liberated is decomposed into ammonia and carbon dioxide. Traces of the acid escape this change, and hence the carbon dioxide evolved has an extremely pungent odour resembling that of sulphurous acid, and most powerfully affects the eyes. The odour is slowly but very well developed by treating a solution of the cyanate with acid tartrate of potassium.[2]

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[1] Four parts of well-dried potassium ferrocyanide in powder is mixed with three parts of dry pulverised potassium bichromate. The mixture is gradually introduced, in small quantities at a time, into a large iron crucible, which is heated to a point just short of red-heat. Oxidation takes place, and is indicated by a glowing of the mass introduced. As soon as one portion has ceased to glow, the next is introduced, and so on, the contents of the crucible being stirred with an iron spatula. The temperature must not be allowed to rise high enough to effect the melting of the resulting mass, which should remain porous and spongy. After cooling, the product is powdered and extracted with three times its weight of boiling methylated spirit of 80 per cent. strength. The solution is filtered, and on cooling deposits potassium cyanate as a perfectly white crystalline powder. The mother-liquor is used to extract the product a second time, and this proceeding is repeated until no further crystals are deposited on cooling. The crystalline powder is then washed several times with small quantities of ether.

[2] Acetic acid, and some other acids, when added to a solution of potassium cyanate, throw down a crystalline precipitate of acid potassium cyanurate, $KH_2C_3N_3O_3$. On adding ether and strong hydrochloric acid to a solution of potassium cyanate, some of the liberated cyanic acid is polymerised to cyanuric acid, which dissolves in the ether.

The ammonia formed in the reaction may be determined by distilling the liquid with slaked lime or caustic alkali. It is evident that these reactions are insufficient for the recognition of a cyanate, when, as in commercial potassium cyanide, a carbonate and cyanide are also present. The detection of cyanate under these conditions is described on page 454.

Cyanates are not affected by alkaline solution of hypochlorites or hypobromites (compare page 274).

The determination of cyanate, when existing in the form of pure potassium cyanate, may be effected by the following process devised by the author:—One gramme of the sample is dissolved in very cold water, and precipitated without delay by an excess of a neutral solution of silver nitrate. The precipitate of argentic cyanate is filtered off and washed slightly. It is then dissolved in a moderate excess of warm normal nitric acid, when the following reaction takes place:—

$$AgOCN + 2HNO_3 + H_2O = AgNO_3 + NH_4NO_3 + CO_2.$$

Hence, one atom of cyanate will neutralise two of nitric acid. The liquid is filtered from any trace of insoluble matter and titrated with normal alkali. Each c.c. of normal nitric acid neutralised by the cyanate represents ·1622 gramme of KOCN in the sample taken. The author obtained 101·6 and 101·2 per cent. in two experiments made by this process on pure potassium cyanate. Probably the same process might be applied to the determination of cyanate in commercial potassium cyanide, the carbonate being first separated by precipitating the cold solution with barium or calcium nitrate. As the cyanide and any chloride which may be present will be precipitated as silver salts together with the cyanate, the subsequent treatment with warm dilute nitric acid will leave an insoluble residue, which must be separated before titrating with alkali.

AMMONIUM CYANATE, $(NH_4).O.CN$, is obtained by mixing cyanic acid vapours with ammonia in excess, when it is deposited in minute crystals which effervesce with acids. The salt may also be obtained by decomposing silver cyanate by a solution of ammonium chloride; or barium, lead, or potassium cyanate by ammonium sulphate (compare page 249). Ammonium cyanate is extremely unstable, being converted by boiling or evaporating the solution into urea, probably with previous conversion into the isocyanate:—$NH_4.O.CN = NH_4.N.CO = NH_2.CO.NH_2$. (This interesting change is fully discussed on pages 249, 251, and 273.)

BARIUM CYANATE, $Ba(CyO)_2$, separates in crystals on mixing alcoholic solutions of barium acetate and potassium cyanate.

Calcium Cyanate, $Ca(CyO)_2$, has been recently manufactured on a large scale.[1] Owing to its high content of nitrogen, and ready decomposition with formation of ammonia, it has been proposed to employ calcium cyanate as a fertiliser.

Cobalt Potassium Cyanate, $Co(CyO)_2,2KCyO$, is precipitated in dark blue quadratic crystals on adding a solution of cobalt acetate (or cobalt nitrate with potassium acetate) to a solution of potassium cyanate. The reaction has been applied to the detection of cyanate in commercial potassium cyanide (see page 454).

Lead Cyanate, $Pb(CyO)_2$, is a crystalline salt, nearly insoluble in hot water. Its preparation is described on page 249.

Silver Cyanate, $AgCyO$, forms a crystalline precipitate, somewhat soluble in boiling water, but 100 parts of water at 16° C. dissolve only 0·006 part of the salt. Dilute nitric acid decomposes it with evolution of carbon dioxide and formation of silver and ammonium nitrates (page 484).

Polymers of Cyanic Acid.

Besides *cyamelide* $(CNHO)_n$, which may possibly be identical with isocyanuric acid, various other polymers of cyanic acid exist, and are known either in the free state or as salts. Thus :—

Fulminic Acid, $H_2C_2N_2O_3$, is unknown in the free state, but the metallic *fulminates* are explosive salts typified by fulminating mercury, used in the preparation of percussion caps.[2]

Mercuric Fulminate, $HgC_2N_2O_3$, is obtained by warming alcohol

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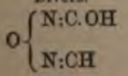
[1] A mixture of limestone and coke is submitted to a preliminary temperature of 1500° in an electric blast-furnace, and is then superheated in the same furnace to 2500° in presence of a large excess of pure nitrogen, and then finally oxidised by means of air, the oxygen of which is retained by the product whilst the nitrogen conveys the heat due to the oxidation into the electric chamber. The operation must be conducted in a large furnace, so that the calorific yield may be sufficiently economical.

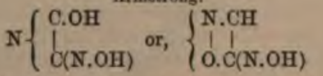
[2] The constitutional formula of fulminic acid is not known with certainty. The following are the formulæ suggested by E. Divers and H. E. Armstrong (*Jour. Chem. Soc.*, xlvii. 79):—

Divers.



$$O\left\{\begin{matrix}N{:}C.OH\\ N{:}CH\end{matrix}\right.$$

Armstrong.



$$N\left\{\begin{matrix}C.OH\\ |\\ C(N.OH)\end{matrix}\right. \text{ or, } \left\{\begin{matrix}N.CH\\ |\quad |\\ O.C(N.OH)\end{matrix}\right.$$

The question has been recently reviewed by R. Scholl (*Ber.*, xxiii. 3505; *Chem. Centralb.*, 1893, i. 730), who considers, with Steiner, that the properties and modes of formation of the fulminates are best explained on the assumption that fulminic acid has the constitution of dioximido-ethylene ;— OH.N:C:C:N.OH (see also F. Holleman *Ber.*, xxiii. 2298, 3742).

with mercuric nitrate and nitric acid. It forms small silky crystals which explode with great violence when heated or struck. Silver fulminate is still more explosive. Concentrated hydrochloric acid decomposes the fulminates with formation of hydroxylamine hydrochloride and evolution of carbon dioxide.

Fulminuric Acid, $HC_3H_2N_3O_3$, results from the action of a chloride or iodide of an alkali-metal on mercuric fulminate.

Cyanuric Acid, $H_3C_3N_3O_3$ or $(CN)_3(OH)_3$. This body is formed by the dry distillation of uric acid, by the action of heat on urea (page 250), and in various other reactions (page 481). Cyanuric acid is a compound of great theoretical interest, as also are the alkyl salts of its isomer *isocyanuric acid,* $(CO)_3(NH)_3$.

THIOCYANATES. SULPHOCYANIDES.[1]

These salts are the sulphur-analogues of the cyanates or oxycyanides. Like cyanic acid, thiocyanic acid exists in two isomeric forms, which are known in their metallic and alkyl salts, though only one modification (probably the normal) of the free acid has been isolated. The ordinary metallic thiocyanates are the normal salts, of the constitution MS.CN, but the isothiocyanates have also been prepared.[2] The normal salts of the alkyl-radicals are known, but they change with great facility into the isomeric forms, which are preferably called thiocarbimides. The type of these compounds is allyl thiocarbimide, the volatile oil of mustard, which has been described on page 107.

A polymeride of thiocyanic acid is known $(H_2S_2N_2C_2)$, and the methyl ether of trithiocyanic acid (thiocyanuric acid), $H_3C_3N_3S_3$, has been obtained.

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[1] These salts are also called sulphocyanates, but such a term would be more appropriately applied to compounds of the formula $M(SO_2)CN$.

[2] Potassium Isothiocyanate. Potassium Thiocarbimide. K.NCS.—This salt is the type of the metallic isothiocyanates. It is obtained by heating perthiocyanic acid with alcoholic potash. The crystals are soluble in water. Potassium isothiocyanate is partially transformed into the normal salt by repeatedly evaporating its solution, and by fusion undergoes complete conversion. The reactions of the isothiocyanates differ from those of the normal salts in many respects. Thus, the silver salt is light yellow, and but slightly soluble in ammonia. With zinc chloride, thiocyanates give no reaction, but the isothiocyanates give a voluminous deep yellow precipitate, and with neutral ferric chloride a brown coloration, disappearing on addition of an excess of the iron salt. Cupric sulphate gives a greenish-yellow precipitate.

Selenium forms a series of salts, selenocyanides, precisely analogous to, but less stable than, the sulphocyanides.[1]

Notable quantities of thiocyanates occur naturally in the saliva and contents of the stomach (page 450), and they may also be detected in the urine. Hence thiocyanates appear to pass through the system unaltered. In the saliva their presence is directly indicated by the red coloration produced on addition of ferric chloride. For the detection of thiocyanates in urine the liquid is precipitated by baryta-water, the filtrate evaporated to a syrup, extracted with alcohol, the solution so obtained again evaporated, the residue redissolved in water, the solution decolorised by animal charcoal, and tested by ferric chloride, &c.

Sinapine thiocyanate exists ready-formed in the seeds of mustard and some allied plants (page 104), and the isothiocyanates of acrinyl and allyl are formed by the action of the ferment myrosin on the glucosides of white and black mustard respectively, while other cruciferous seeds yield identical or analogous compounds (page 101).

Thiocyanates are also present in notable quantity in the ammoniacal liquor and spent oxide obtained in the purification of coal gas, and in the liquors resulting from the lixiviation of the black-ash produced in the Leblanc process of manufacturing caustic soda. The recovery of sulphocyanides from these and allied sources and their manufacture on the large scale by various synthetical methods with a view to conversion into cyanides or ferrocyanides have formed the subject of various patents[2] (see page 450).

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[1] *Silver Selenocyanide*, $AgSeCN$, is a white curdy precipitate closely resembling the sulphocyanide. The *cuprous salt* is white and insoluble. On adding a drop of ferric chloride to a cold dilute solution of a selenocyanide, a red coloration is momentarily produced, probably owing to the formation of *ferric selenocyanide*, but the solution very rapidly becomes turbid and free selenium separates.

[2] Interesting descriptions of the manufacture of sulphocyanides from these sources will be found in the *Chem. Zeitung* for 1886 (abst. *Jour. Soc. Dyers*, &c., ii. 42, 85), and in a paper by J. V. Esop (*Zeits. angew. Chem.*, 1889, 305; abst. *Jour. Soc. Chem. Ind.*, viii. 881), who also gives analyses of various specimens of spent oxide. In a process patented by H. Bower (*Eng. Patent*, No. 8330, 1895), gas-liquor is distilled, after addition of iron or an iron salt and of lime, to remove ammonia, the cyanogen present being left in the liquor as calcium ferrocyanide and sulphocyanide. An acid solution of a copper salt, preferably cuprous chloride, is added to obtain a mixed precipitate of copper ferrocyanide and sulphocyanide, which is separated. The moist precipitate is agitated with finely divided iron, to produce copper and iron ferrocyanide and soluble iron sulphocyanide. The iron ferrocyanide, on treatment with an alkali, yields a soluble ferrocyanide; while the solution of iron sulphocyanide may be concentrated and crystallised, or evaporated to dryness.

In the process of Gélis, as modified by Günzberg and Tscherniac, carbon disulphide and ammonia of 20 per cent. strength are caused to react in a closed vessel at 100° C. The product is a solution of ammonium dithiocarbamate mixed with unattacked carbon disulphide. On transferring it to a still and raising the temperature to 110°, the dithiocarbamate splits up into sulphuretted hydrogen and ammonium thiocyanate.[1] The latter remains in the still in aqueous solution, which is concentrated and allowed to stand in tin-lined wooden vessels. Crystals of ammonium thiocyanate are thus obtained.[2] If other cyanogen compounds are required the solution is distilled with lime, which yields a solution of ammonia of suitable strength for treating with a fresh quantity of carbon disulphide, together with calcium thiocyanate.[3] From this salt, potassium thiocyanate is obtained by double decomposition with potassium sulphate. The precipitate is removed by a filter-press, the residual lime thrown down by potassium carbonate, and the filtered liquid evaporated at 125°. On cooling, it deposits the remaining sulphates and chlorides very completely, and nearly pure potassium thiocyanate is obtained on evaporating the solution to dryness.

Potassium thiocyanate results from the action of free sulphur or of certain metallic sulphides on cyanide or ferrocyanide of potassium at a high temperature. It may also be obtained by boiling a solution of potassium cyanide with sulphur, and by several other reactions.

As a class, the thiocyanates are very poisonous,[4] and have as deleterious an effect on plants as on animals (see page 496).

Most of the metallic thiocyanates are soluble, the chief exceptions being the cuprous, mercurous, mercuric, lead, and silver salts. From a solution acidulated with hydrochloric acid and filtered, thiocyanates precipitate copper salts only. The thiocyanates readily form double salts.

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[1] $CS_2 + 2NH_3 = CS(NH_2)SH,NH_3 = H_2S + NH_4.SCN$.

[2] Mere traces of iron cause the salt to become red on exposure to air, but the iron may be readily removed from the solution by addition of ammonium sulphide, or by ammonia in presence of air. The filtered liquid must be evaporated in a tin vessel.

[3] The Günzberg-Tscherniac process of manufacturing calcium thiocyanate is less economical than that of Hood and Salamon and the supplementary patents which are described in outline on page 450.

[4] In a case where a woman took ammonium sulphocyanide she was found unconscious, with rigidity of the muscles of the arms and jaws. In spite of attempts to revive her, convulsions ensued, followed by death in sixteen hours from the first symptoms observed.

A dose of five grains of ammonium thiocyanate is said to be immediately fatal.

DETECTION OF THIOCYANATES.

The following reactions of analytical and general interest are given by a solution of potassium or other soluble thiocyanate :—

On addition of a mineral acid dilute solutions of thiocyanates suffer no immediate change, but strong solutions are decomposed with formation of thiocyanic acid and other products, and separation of perthiocyanic acid (see P. Klason, *Jour. prakt. Chem.*, [2], xxxvi. 57 ; abst. *Jour. Chem. Soc.*, 1887, page 1025).

On distillation with dilute sulphuric acid, potassium thiocyanate yields thiocyanic (hydrosulphocyanic) acid, HSCN, as a liquid of a pungent odour. Part of the acid is decomposed in a manner analogous to cyanic acid :—$2HSCN + 2H_2O = 2H_3N + CS_2 + CO_2$. Another portion is split up thus :—$3HSCN = HCN + H_2C_2N_2S_3$.[1]

From a hot solution of potassium thiocyanate, nitric acid, chlorine, or a mixture of hydrochloric acid and potassium chlorate precipitate a yellow substance containing $C_3HN_3S_3$, inaptly called perthiocyanogen.[2]

Silver nitrate precipitates from solutions of soluble thiocyanates white curdy argentic thiocyanate, AgSCN, insoluble in dilute nitric acid, but soluble in ammonia and in soluble thiocyanates.

Cupric sulphate produces no immediate change in a weak solution of thiocyanates, but in a strong solution precipitates black cupric thiocyanate, $Cu''(SCN)_2$, which turns white on standing. If sodium sulphite, sulphurous acid, or other reducing agent be added together with the cupric solution, a white precipitate, consisting of cuprous thiocyanate, $Cu'_2(SCN)_2$, is immediately formed. The precipitate is insoluble in water and saline solutions, and nearly insoluble in dilute sulphuric and hydrochloric acids, but dissolves in ammonia.

Ferrous sulphate, if quite free from ferric salt, occasions no change in solutions of thiocyanates.

When added to a slightly acid solution of a soluble thiocyanate, ferric sulphate or chloride produces a deep red coloration, owing to the formation of soluble red **ferric thiocyanate**, $Fe(SCN)_3$. This is a most delicate and characteristic reaction for ferric salts and thiocyanates. The colour is not destroyed by boiling, or by cold dilute mineral acids (distinction from acetates and formates). The fixed alkalies and ammonia precipitate brown ferric hydroxide, and thus destroy the colour.[1] The colour is instantly destroyed by mercuric chloride (distinction from meconates) or by excess of silver nitrate (distinction from formates and acetates). In presence of ferrocyanide, excess of ferric solution should be added and the liquid filtered from the precipitate of prussian blue, when the red colour will become apparent. In presence of ferricyanide, the dark-coloured solution should be largely diluted.

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[1] The characters and decompositions of thiocyanic acid have been investigated by P. Klason (*Jour. prakt. Chem.*, [3], xxxv. 789 ; abst. *Jour. Chem. Soc.*, 1887, page 789).

[2] PERTHIOCYANOGEN, $C_3HN_3S_3$, is an amorphous, deep yellow substance, insoluble in water, alcohol, or ether, and unaffected by dilute alkalies or acids. It has been applied, under the name of "canarin," for printing calico, the colour being formed in the fibre, and developed in a manner similar to aniline black. Perthiocyanogen acts as a mordant to many of the coal-tar dyes, such as methylene-blue, aniline-green, aniline-red, &c. (see *Dingler's polyt. Jour.*, ccli. 41 ; abst. *Jour. Chem. Soc.*, 1884, p. 796).

The **author** has observed that when the red liquid produced by adding a ferric salt to a soluble thiocyanate is shaken with ether, the colour passes wholly into the ether if the thiocyanate be present in excess; but if the iron salt be in excess the ether remains uncoloured. By evaporation of the aqueous or ethereal solution of ferric thiocyanate, intensely deep red crystals are obtained, readily soluble in water, alcohol, and ether.

According to **Krüss** and **Moraht** (*Ber.*, xxii. 2061) the red coloration produced by adding potassium thiocyanate to a ferric salt is due to the formation of a compound containing $Fe(SCN)_3,9KSCN + 4H_2O$. This salt may be obtained by adding the calculated quantity of potassium thiocyanate to a neutral solution of ferric thiocyanate. It is insoluble in dry ether, but is decomposed by moist ether into potassium thiocyanate, which is insoluble, and ferric thiocyanate, which dissolves in the ether with red colour. A compound containing $Fe(SCN)_3,3KSCN$ has also been obtained.

The red coloration produced on mixing solutions of ferric salts and thiocyanates cannot be employed for colorimetric determination of either body, since the intensity varies in a complex manner with the temperature and concentration of the liquid, the relative proportions of iron and thiocyanate, and the form in which they are added. Even when 12 molecules of thiocyanate are present for 1 of ferric salt, the maximum coloration is not obtained.

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[1] The thiocyanate reaction is not obtained in a solution of a ferric salt to which sodium acetate has been added, until the solution is strongly acidified with hydrochloric acid. Nor does the test answer for solutions of basic ferric salts, obtained by digesting dilute ferric chloride with ferric hydroxide, or by adding ammonium carbonate to ferric chloride solution as long as the precipitate is redissolved. Very dilute ferric solutions free from acidity are inactive towards thiocyanate solutions at ordinary temperatures, and somewhat stronger solutions do not give the colour when hot. This is explained by the complete hydrolytic dissociation which occurs in dilute solutions at ordinary temperatures, and in stronger solutions at higher temperatures. Acidulation of the solution, however, always renders the thiocyanate test applicable.

A solution of molybdic acid or molybdate of ammonium in hydrochloric acid gives a red colour with a thiocyanate which, like the similar colour produced by ferric salts, is removed from its aqueous solution by agitation with ether.

When a thiocyanate is treated with zinc and hydrochloric acid, sulphuretted hydrogen is evolved (which will blacken lead paper held over the tube), and methylamine, $(CH_3)H_2N$, is formed in the solution.

When added to a solution of a thiocyanate acidulated by dilute hydrochloric acid, a solution of potassium permanganate is instantly decolorised with formation of hydrocyanic and sulphuric acids.

Various kinds of organic matter give a red colour with soluble thiocyanates. The effect has been shown by C. Parenti (abst. *Jour. Chem. Soc.*, 1890, p. 726) to be due to the presence of traces of ferric compounds.

DETERMINATION OF THIOCYANATES.

The reaction with permanganates is one of the most convenient for the determination of thiocyanates, and may be applied either gravimetrically or volumetrically.

1. For the gravimetric determination, any sulphate is removed by treating the cold solution with dilute hydrochloric acid and excess of barium chloride. The filtrate is treated with a slight excess of potassium permanganate, and more barium chloride is added if necessary. 233 parts of $BaSO_4$ precipitated represent 58 of thiocyanogen, SCN. The method is applicable to all thiocyanates soluble in water or dilute acids. It is not interfered with by chlorides, but is inapplicable in presence of sulphites, thiosulphates or sulphides. The last class of compounds may be previously removed by a solution of a cadmium salt.

H. Alt (*Ber.*, xxii. 3258) proposes to oxidise the thiocyanate with nitric acid instead of permanganate, but the advantage is not obvious.

2. In the absence of other reducing agents, thiocyanates may be directly titrated in the cold with dilute sulphuric acid and standard permanganate. The reaction is as follows:—$5KSCN + 6KMnO_4 + 12H_2SO_4 = 11KHSO_4 + 6MnSO_4 + 5HCN + 4H_2O$; or, more simply:—$HSCN + O_3 = HCN + SO_3$.

Each c.c. of decinormal permanganate decolorised represents

0·00193 gramme of SCN.[1] The solution should, however, be standardised by pure potassium or ammonium thiocyanate.

Thiocyanates may be determined by the above method in presence of simple cyanides and chlorides. For the determination of the simple cyanide in such a mixture, the solution should be treated with excess of silver nitrate and the washed precipitate treated by Kjeldahl's process, metallic mercury being added and fuming sulphuric acid substituted for acid of 1·84 specific gravity. The ammonia formed is derived from the cyanide and thiocyanate, and the latter having been previously determined by titration with permanganate the cyanide can be deduced. The chloride can be determined by difference, or directly estimated by oxidising the solution with permanganate in a solution acidulated with sulphuric acid, boiling off the hydrocyanic acid, preferably in presence of granulated zinc, and then precipitating the chloride with silver nitrate.[2]

3. Volhard's process for the determination of silver is equally applicable to the titration of thiocyanates. The solution of the thiocyanate is acidified with nitric acid (previously well boiled to

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[1] According to P. Klason (*Jour. prakt. Chem.*, xxxvi. 74), the results obtained by titration with permanganate are always too low, the error being greater as the solution is more dilute. When the concentration is not less than decinormal, the result is about 1½ per cent. below the truth, and a corresponding correction can be made.

[2] For the determination of cyanides, thiocyanates, ferrocyanides, and ferricyanides when the four classes of salts occur together in solution without heavy metals, the following method may be used :—A measured quantity of the liquid is strongly acidulated with hydrochloric acid, and precipitated with excess of ferric chloride. The precipitate of prussian blue is filtered off, washed, boiled with caustic alkali, and the alkaline *ferrocyanide* produced filtered from the precipitated ferric hydroxide and determined by titration with standard permanganate, or by one of the other methods described on page 466 *et seq.* The filtrate from the ferric chloride precipitate is treated with ferrous sulphate and the liquid again filtered. The precipitate consists of ferrous *ferricyanide*, which on boiling with caustic alkali yields soluble ferrocyanide as described on page 475. The filtrate from the precipitate produced by ferrous sulphate, which must contain excess of both ferrous and ferric salts, is treated with excess of soda and heated. It is then again acidified with hydrochloric acid, when a precipitate of prussian blue will result, the amount of which represents the *simple cyanide* originally present. It may be boiled with alkali, and the ferrocyanide produced titrated as before, and calculated to Cy. The *thiocyanate* may be determined in the filtrate from the various iron precipitates by precipitation as cuprous salt, cupric sulphate, or in a separate portion of the original liquid by oxidising it in acid solution with permanganate, and precipitating the resultant sulphate by barium chloride.

free it from nitrous acid), and a solution of ferric sulphate added. This produces a deep red solution of ferric thiocyanate. Decinormal silver solution is next added from a burette until the red colour is replaced by a light brown and the latter is at last destroyed. The end-reaction is better observed by adding excess of silver solution and titrating back with standard thiocyanate until a light brown tint is permanent on agitation. Each c.c. of decinormal silver nitrate used represents 0·0058 gramme of SCN. In presence of ferrocyanides, excess of iron solution must be added and the liquid filtered before titrating with silver nitrate. The same plan is applicable in presence of ferricyanides if ferrous sulphate be substituted for the ferric salt. Cyanides may also be removed by ferrous and ferric salts and alkali, with subsequent acidification by dilute nitric acid, followed by filtration. Sulphides may be separated by filtering the solution *after* addition of iron salts and alkali, but *before* adding nitric acid. In presence of chlorides, bromides, or iodides, the process is still available, if, after the termination of the reaction, the silver precipitate be filtered off and treated in the following manner:—The silver compound is dried and mixed with a large excess of pure sodium carbonate. The mixture is added gradually to fused nitre contained in a porcelain crucible. When the action is complete, the cooled mass is dissolved in water. The filtered liquid is neutralised by dilute nitric acid and titrated with silver nitrate, using neutral potassium chromate as the indicator. The silver solution used represents the chloride, bromide, and iodide present. Its volume, deducted from the amount originally required, represents the silver solution corresponding to the thiocyanate. The thiocyanate may also be deduced from the amount of sulphate formed on fusion with nitre. This plan does not necessitate the previous removal of cyanides, ferrocyanides, or ferricyanides.

4. Any sulphide, cyanide, ferrocyanide, or ferricyanide is separated by iron salts, as in process 3, but the liquid is rendered slightly acid by hydrochloric acid instead of by nitric acid. The solution is then treated with sodium sulphite and a solution of cupric sulphate added. Phosphates and other inorganic salts forming insoluble copper compounds may be got rid of by digesting the precipitate with cold dilute hydrochloric acid. The white precipitate of cuprous thiocyanate is filtered off, washed, dried at 100° C. and weighed. 121·3 parts of $Cu_2(SCN)_2$ represent 58 of thiocyanogen. The process is not affected by bromides or chlorides, but is not directly applicable in presence of iodides, which are precipitated as white cuprous iodide. If the solution be acidulated with sulphuric acid instead of hydrochloric acid

before precipitating the thiocyanate as a cuprous salt, any chloride or bromide can be determined in the filtrate as a silver salt in the usual manner.

The precipitate of cuprous thiocyanate is sometimes so finely divided that a clear filtrate is extremely difficult to obtain. This is especially the case in the presence of thiosulphates and certain other salts, such as co-exist with thiocyanates in gas-liquor. For the determination of thiocyanates in such cases, S. Dyson (*Jour. Soc. Chem. Ind.*, 1883, page 231) recommends that 50 c.c. of the sample should be evaporated to dryness and the residue heated to 100° for three or four hours. If this prolonged heating be omitted, the cuprous thiocyanate will be precipitated in such a finely-divided state as to render filtration almost impossible. The residue is digested with strong alcohol, rinsed on to a filter and washed with alcohol. Thiosulphates, which otherwise exercise a solvent action on cuprous thiocyanate, are left undissolved. The alcoholic filtrate is evaporated to dryness, the residue taken up with water, and the insoluble organic matter filtered off. A solution of ammonium thiocyanate is thus obtained tolerably free from other ammoniacal salts and from organic matter. The solution is then treated with sulphurous acid and cupric sulphate, gently warmed (not boiled), and the precipitate of cuprous thiocyanate filtered off.

A volumetric modification of the copper method of determining thiocyanates has been described by Barnes and Liddle (*Jour. Soc. Chem. Ind.*, 1883, page 231). It consists in boiling the solution with sodium bisulphite, and titrating with a standard solution of copper sulphate, the termination of the reaction being indicated by the immediate production of a brownish colour on bringing a drop of the liquid in contact with a drop of solution of potassium ferrocyanide on a white plate. The results are liable to several disturbing influences, but the process is useful under favourable conditions.

5. Very small quantities of thiocyanates, such as are met with in soda-lyes, may be determined by acidifying the lye with hydrochloric acid, and adding zinc chloride. Any ferrocyanide of zinc is filtered off, and the filtrate coloured by ferric chloride. The tint is then compared colorimetrically with that produced by a known quantity of thiocyanate treated similarly. The results are only roughly approximate.

6. *Insoluble thiocyanates* may be fused with alkaline carbonate and nitre, as in process 3, the sulphate produced being determined by precipitation with barium chloride. They may also be decomposed by sulphuretted hydrogen, the liquid filtered,

ammoniacal solution of copper added, the sulphide of copper filtered off, and the filtrate treated with dilute sulphuric acid and sodium sulphite, when cuprous thiocyanate will be obtained as in process 4.

J. V. Esop (*Jour. Soc. Chem. Ind.*, 1889, p. 881) has observed that the thiocyanates contained in the spent oxide from gas-purifiers are not entirely soluble in water, but can be completely dissolved out by alkali. The presence of other sulphur compounds renders the oxidation process quite inapplicable. The alkaline extract is best treated by process 5.

7. To determine the *metals* in thiocyanates, the salts may be decomposed by sulphuric acid as described on page 441.

POTASSIUM THIOCYANATE. POTASSIUM SULPHOCYANIDE. KS.CN.

This salt crystallises in colourless, anhydrous, deliquescent needles, very soluble in water and alcohol. The aqueous solution gradually decomposes at ordinary temperatures and more rapidly when boiled, with evolution of ammonia. The solution dissolves argentic chloride, cyanide, and thiocyanate.

When strongly heated without contact of air, potassium thiocyanate evolves carbon disulphide and leaves a residue containing potassium sulphide and potassium mellonide, $K_3C_9N_{13}$, which crystallises with 3 aqua from hot water.

The analytical reactions of potassium thiocyanate are described on page 489 *et seq.*

AMMONIUM THIOCYANATE. AMMONIUM SULPHOCYANIDE. $(NH_4)SCN$.

This salt is produced by various reactions (see method for detecting hydrocyanic acid, page 429), including the action of carbon disulphide on ammonium sulphide :—$(NH_4)_2S + CS_2 = NH_4SCN + 2H_2S$ (compare page 488). Ammonium thiocyanate is a product of the distillation of coal, and is found in considerable quantities in the ammoniacal liquor and spent oxide of the gas-works. The salt forms colourless deliquescent plates which are very soluble in water and alcohol, the solution taking place with great reduction of temperature. The dilute aqueous solution is stated to be perfectly stable.

Ammonium thiocyanate melts at 159°. When kept nearly at this temperature for some time, it suffers conversion into thio-urea :— $NH_4.S.CN = NH_2.CS.NH_2$. At 180° to 190° it evolves sulphuretted hydrogen, carbon disulphide, and ammonia, and leaves a residue containing guanidine thiocyanate (compare page 283). At a still higher temperature melam, $C_6H_9N_{11}$, is obtained.

The reactions of ammonium thiocyanate in solution are strictly analogous with those of the potassium salt (see page 489).

Commercial sulphate of ammonium often contains thiocyanate, which prejudicially affects its application as a fertiliser. P. L. Jumeau (*Analyst*, xviii. 135) has described a specimen which contained 9 per cent. of ammonium thiocyanate and a similar proportion of sodium sulphate, but such samples are very unusual. In determining the amount of thiocyanate, it is desirable, though not essential, to extract the sample with methylated spirit, and evaporate an aliquot part of the solution, and determine the thiocyanate in the residue, by one of the methods given on page 491.

Ammonium thiocyanate acts as a powerful poison to animals (page 488) and also exerts a toxic action on plants. According to Mack and Silen, maize is particularly sensitive to its action, as small a quantity as 9 kilogrammes of thiocyanate per hectare showing its influence in the generally deteriorated appearance of the plants. G. Klein found that, when watered with a solution containing 0·01 gramme of ammonium thiocyanate per litre, old plants with six to eight leaves were uninjured, but sickened at once with a solution of twice the strength, and 0·1 gramme per litre proved fatal almost immediately. Seeds lose their power of germination when steeped in ¼ per cent. solution of ammonium thiocyanate.

BARIUM THIOCYANATE, $Ba(SCN)_2$, is now produced on a large scale by boiling ammonium thiocyanate with baryta-water, or by decomposing cuprous thiocyanate (from spent oxide or gas-liquor) with barium sulphide. It forms long deliquescent needles, commonly stated to contain 2 aqua (but according to J. Tscherniac, 3 aqua), and very soluble in water and alcohol. Its chief application is for the manufacture of aluminium thiocyanate.

CALCIUM THIOCYANATE, $Ca(SCN)_2$, forms very soluble, deliquescent needles containing 3 aqua. It is prepared on a large scale by several patented processes (see page 488).

ALUMINIUM THIOCYANATE, $Al(SCN)_3$, is obtained by the double decomposition of aluminium sulphate with barium or calcium thiocyanate (see *Jour. Soc. Chem. Ind.*, i. 64, 364). Any red colour due to the presence of traces of iron can be removed by agitation with ether. Aluminium thiocyanate now receives extensive application in calico-printing.[1] A soluble basic sulphocyanide of aluminium, containing $Al(OH)_2(SCN)$, is obtained by dissolving hydrated alumina in aluminium thiocyanate.

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[1] The sulphocyanides of aluminium and other metals are used in dyeing and calico-printing for three distinct purposes :—as a resist for aniline black ; as an addition to the ordinary alizarin-red printing colour in order to resist the action of iron ; and as the mordant for alizarin-red instead of the acetates (see Lauber and Storck, *Jour. Soc. Chem. Ind.*, 1882, page 359). When applied to the last purpose thiocyanates are found to produce greater brilliance of tint and fastness on the fibre, apparently from the gradual manner in which they undergo decomposition on steaming.

Cuprous Thiocyanate, $Cu_2(SCN)_2$, is one of the most insoluble salts of the series. Its properties are described on page 489. Cuprous sulphocyanide occurs in commerce under the name of "white paste," which is obtained by acting on ammonium thiocyanate with a mixture of cupric and ferrous sulphates. A 47 per cent. paste is commonly sold. It should be tested for iron, barium sulphate, and soluble matters, and the thiocyanate determined by boiling the paste with caustic soda, acidulating the filtrate with dilute sulphuric acid, and titrating with permanganate (page 491).

Lead Thiocyanate, $Pb(SCN)_2$, forms a yellowish-white crystalline precipitate, somewhat soluble in hot water and readily soluble in water acidulated with hydrochloric acid.

Mercuric Thiocyanate, $Hg(SCN)_2$, is obtained as a sparingly soluble, white, crystalline precipitate on adding a soluble thiocyanate to a strong solution of mercuric nitrate or chloride. It is soluble in excess of the precipitant and in dilute hydrochloric acid. On heating mercuric thiocyanate in a test-tube, or on kindling the powder, it ignites and swells up enormously, giving off sulphur dioxide, mercurial vapours, &c., and leaving a very bulky porous grey or brown mass containing mellon, $C_3H_3(NH)_3C_3H_3$.

"Pharaoh's serpents' eggs" consist of mercuric thiocyanate.[1] On treatment with hot water they yield a solution which gives a yellow precipitate with caustic alkali. On filtering off the precipitated mercuric oxide, the filtrate gives the reactions of a thiocyanate after being acidulated with dilute nitric acid.

Several cases of poisoning have occurred owing to children swallowing "Pharaoh's serpents' eggs."

Ferric and *argentic thiocyanates* have already been described (page 489).

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[1] Not of mercurous thiocyanate, as erroneously stated in some text-books. Mercurous thiocyanate is extremely unstable, rapidly becoming grey from the separation of metallic mercury.

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ERRATA AND ADDENDA.

VOLUME I.

Page 71, line 20, *for* "46," *read* "92."

Page 74, *for* "2 $C_2H_6O_2$," *read* "2 C_2H_6O."

Page 15, line 20, *for* "pressure," *read* "presence."

Page 121, line 17, after word "is," *insert* "then washed thoroughly by repeated agitation with water, to remove ethylic alcohol and"

Page 230, line 18, after the words "side by side," *insert* "with that."

VOLUME II.

Page 417, line 1, *for* "$C_{16}H_{24}$," *read* "$C_{15}H_{24}$."

VOLUME III. PART I.

Page 193, in heading of penultimate column, *for* "of," *read* "and."

Page 264, footnote, *for* "$C_{26}H_{24}O_{14}$," *read* "$C_{26}H_{28}O_{14}$."

VOLUME III. PART II.

Page 17, in Table, *for* "Boiling point, 0° C," *read* "boiling point, °C."

Page 81, in formula of phenacetin, *for* "(C_2H)," *read* "(C_2H_5)."

Page 106, chemistry of piperazine and spermine, *see* Part iii. page 194, *et seq.*

Pages 109 and 112, *for* "Hautsch," *read* "Hantzsch."

Page 179, line 7, the formula for lupanine should be "$C_{15}H_{24}N_2O$."

Page 218, line 2 of footnote, *delete bracket* and word "see"; and in following line *insert* bracket after first comma.

Page 219, *for* "colouring changed," *read* "colour changing."

Page 274, line 29, *for* "say," *read* "ray."

Page 403, in footnote, *for* "0·0324," *read* "0·0162"; and compare Part iii. page 81, footnote.

Page 484, on the Determination of Caffeine in Tea, see papers by E. H. Gane (*Jour. Soc. Chem. Ind.*, 1896, p. 95) and Petit and Terrat (*Pharm. Jour.*, [4], ii. 461).

Page 499, line 4, *for* "$C_7H_7A_9N_4O_2$," *read* "$C_7H_7A_gN_4O_2$."

Page 578, *insert* "Kola, 554."

VOLUME III. PART III.

Page 25, on Brazilian and Columbian Ipecacuanha, see a paper by Paul and Cownley (*Pharm. Jour.*, [4], ii. 321).

Page 65, line 13 from foot, *insert* "that" *after* "out."

Page 130, see a paper "On the Detection of the Digitalis Glucosides and their Products," by H. Kiliani (*Archiv. der Pharm.*, ccxxxiv. 273; and *Pharm. Jour.*, [4], ii. 401).

Page 174, on the Constituents of Lupulin, see H. Seyffert (*Jour. Soc. Chem. Ind.*, June 1896).

Page 180, "On the Tannin of Hops," see J. Heron (*Jour. Soc. Chem. Ind.*, June 1896).

Page 193, line 20, *for* "hypox-anthine," *read* "hypo-xanthine."

Page 214, line 15, after word "from," *insert* "the."

Page 451, Manufacture of Cyanides, see Eng. Patents, 10,476 and 10,956 of 1895.

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